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PHYSIOLOGICAL REVIEWS

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XIIth International Physiological Congress

At the XIth International Physiological Congress in Edinburgh 1923 a committee was appointed to consider and decide on the date and place of the next meeting. The committee consisted of the following: Professors F. BOTTAZZI, W. EINTHOVEN, O. FRANK, E. GLEY, W. H. HOWELL, J. E. JOHANSSON and E. H. STARLING. A meeting of this committee was held on December 31st, 1924, in Paris. In accordance with the decision of the committee the XIIth International Physiological Congress will be held in Stockholm, August 3rd to August 6th, 1926.

Those who desire to be enrolled as members are requested to forward their names and addresses, together with the amount of their subscription (20 Swedish crowns), to Doctor G. LILJESTRAND, Karolinska Institutet, Stockholm, Sweden. Further particulars will be sent upon request.

Stockholm, November 1925

J. E. JOHANSSON,
President of the Congress.

PHYSIOLOGICAL REVIEWS

VOL. VI APRIL, 1926 No. 2

THE PHYSIOLOGY OF THE SPAWNING MIGRATION

CHAS. W. GREENE

Department of Physiology, University of Missouri, Columbia

The migrations of birds have held both scientific and popular interest for many decades. That the spawning migrations of fishes are even more extensive and quite as interesting is not so generally known, for the movements of the finny tribe are invisible beneath the surface of the waters wherein they dwell. A distance of one or two thousand miles may separate the feeding grounds of the adults from the beds wherein the spawn is laid and the life of the young takes origin. Some, hatching in the ocean, make the trip to fresh water in their youth, and after full growth and development in the rivers and lakes, journey back to the briny deep. Other fishes make the same circle but in the opposite direction.

Salmo salar of the Rhine has furnished a classical example of the latter. The eggs are deposited in the cold waters of the Alpine streams and the embryonic stages are passed in such waters. The young fry migrate to the sea during the spring and summer freshets of the following year. Amid the abundant life and food of the ocean the young salmon develop rapidly and may attain a comparatively enormous size. When three to four years old, the adults find their way back to the mouth of the Rhine in which they were hatched. Once in fresh water again, they cease taking food and the journey to the headwaters is made entirely while fasting. In the mountain streams the adults pair, construct nests in the sand and gravel, and spawn their eggs. Many die but some make their way back to the ocean for further feeding. This is a remarkable life history and in many ways a biological enigma, and it serves to indicate the interest and importance attached to the study of the spawning migration.

An appreciation of the biological significance of such migrations has been possible as the details of the life histories of the different fishes have

been acquired. The securing of accurate and scientific data regarding the life histories of such wide ranging species of fish has been incomparably more difficult than the similar study of birds or other animals. The tremendous commercial importance of the fisheries has greatly stimulated interest in such problems and helped surmount some of these difficulties. Our present knowledge represents the result of the analysis and arrangement of a confusing array of data from widely divergent sources. Much information has been obtained by statistical methods with regard to: 1. The geographical distribution of many species of fishes. 2. The seasonal appearance and disappearance of particular species in regions of great extent and of wide separation. 3. The migratory movements of schools of adults at the pre-spawning and spawning season. 4. The spawning seasons and spawning regions for many migratory species. 5. The development and migration of the young. 6. The rate of growth into adults determined largely from growth rings in the scale structure. 7. The foods, feeding habits and feeding areas. 8. The intensive spawning migration and the nesting habits. 9. The fate of the adults after spawning.

Statistical and group studies have been supplemented by methods of following the individual fishes through different phases of their life histories. Individual fishes have been given identifying marks or numbered tags and released in an effort to follow the course, or speed, or weight changes, or the individual history as written by nature in the growth rings of the scales. Studies of fishes have also been made by the methods of physiological and metabolic experimentation, by biochemical comparison of individuals in different stages of growth and migration, by the chemical study of the reproductive organs in the different stages of development, and by the histological structure of tissues and tissue food deposits in relation to the stages of migration.

THE MIGRATION OF FISHES THAT LIVE AND SPAWN IN THE SEA. Little need be said regarding the habits of those fishes spending their lives wholly in fresh water. Sportsmen and scientists have made many observations in regard to the details of their life history, nest building, spawning habits, etc. The movements and change of habit that have been reported in connection with the spawning period may be passed over in this discussion.

That many marine fishes do not spawn on the feeding grounds frequented by the adults has long been known. J. Schmidt of the Danish fisheries, 1909, was able by statistical methods to show that the spawning grounds of the European cod fish were in the deep waters of the

Atlantic off the continental shelf of the coast of France and Portugal and to the north of the Azores. The adult fish, as shown by the cod fisheries, frequent the waters of the north coast of Europe, Iceland, Scotland, Norway and Denmark.

H. M. Smith marked numbers of adult cod fish as they were released after the egg-taking at the United States fish cultural station at Woods Hole, Massachusetts, during the four seasons beginning December, 1897. He used numbered copper tags and must be credited with the first marking of individual fish in America. Of a total of 4,019 released, 140 were recaptured. By the test Doctor Smith demonstrated that the dispersal migration was over a wide range of the Mid-Atlantic coast as far south as off Atlantic City, New Jersey.

A most unusual and scientifically interesting example of a marine spawning habit was described recently by Thompson for the California smelts, *Leuresthes tenuis*, known as the grunion. During the high tides from March to June large schools of the grunion appear along the sandy beaches of the California coast on the nights when the full lunar tides sweep high up the beaches, and, "for an hour or more after the time of the highest tide the schools work back and forth along the beach." From these schools numerous individuals and spawning pairs of grunion emerge on every wave. They come in on the crest of the waves as they break high up on the sands. The fish lies for a moment glittering in the faint light, then squirms and flops back into the wash of the ebbing wave." The spawning fish are often found stranded on the sand or occasionally "buried tail downward to the level of the pectoral fins" spawning near the highest line reached by the waves.

In the process of spawning the females deposit the eggs two or three inches under the surface of the sand. The males closely attend the females, and when the latter agitates the wet sand the male extrudes the milt and fertilization takes place in the water. The whole spawning process takes only an average of twenty to thirty seconds.

The spawning habit of the grunion is unique among the many species of this family. Its eggs are protected under the warm, relatively dry sand, above the wash of the waves except at very high tides. They are safe from many of the usual agencies that destroy fish eggs and young. Development goes on safely and rapidly. Two weeks later when the next high tide arrives to wet the eggs they are ready to hatch and the embryo quickly bursts its shell and emerges to regain the sea.

The examples of the cod fish and of the grunion will serve as types of migration at the spawning period made by numerous marine species.

These migrations apparently result in more favorable temperature for development, better protection for eggs and embryos, or a richer food supply for the young.

THE SPAWNING MIGRATION OF THE CATADROMOUS FISHES. The catadromous fishes live and feed in the fresh water lakes and streams but return to sea to spawn their eggs. Or perhaps the story had better be reversed, for it would seem that these are true marine fishes that have come to spend a part of their life cycle feeding and developing in fresh water. The common fresh water eel is the famous example of this group. The two common species of eel are *Anguilla vulgaris* in the rivers of northern Europe, and *Anguilla rostrata* in the rivers of the American side of the North Atlantic. These look alike but are easily distinguished by a difference in the number of vertebrae, a characteristic which has also been used to separate the larval forms.

The absolute disappearance of the adult eels from the rivers at a certain season was known even to the ancients, as was also the fact that in early spring countless millions of young eels suddenly appeared. Aristotle thought that these young eels developed out of mud. Absolutely no knowledge of the breeding places or of embryological history was known to early scientists. This was the state of "the eel problem" until late in the 19th century.

From the experiences of the commercial fisheries and an occasional pelagic specimen obtained in scientific exploration the initial course of the migrating adult eels was determined as follows: The adult eels leave the fresh water streams in the spring and early summer and are found along the immediate coast for a few months. Along the European coast they appear in salt water, first off the Coast of Norway and Sweden, later off Denmark and the English channel in a well-marked course of migration. Next they are found along the French and Spanish coast and eels have been taken as far west as the Azores. No further positive observation of the course of the adults is known.

The young eels, called elvers, appear in the early spring months in large numbers along the western European coast pressing in countless myriads through the estuaries and ascending the fresh water streams. These young are between 6 and 7 cm. long and have the form and structure of the adult eel.

The story of the life history of the eel from the disappearance of the adult in the sea to the reappearance of the young elvers has been exceedingly difficult to determine. The first step in its solution was made

when Kaup, 1856, described a very small, elongated, flat and glassy transparent supposed new species under the name of *Brevirostris leptocephalis*. Other similar transparent forms were discovered later until the suspicion arose that these new species were not adults but larval forms. Grassi with Calandruccio, 1896, obtained transition forms in the Mediterranean which proved that *Brevirostris leptocephalis* was the larval form of the common European eel, Anguilla vulgaris.

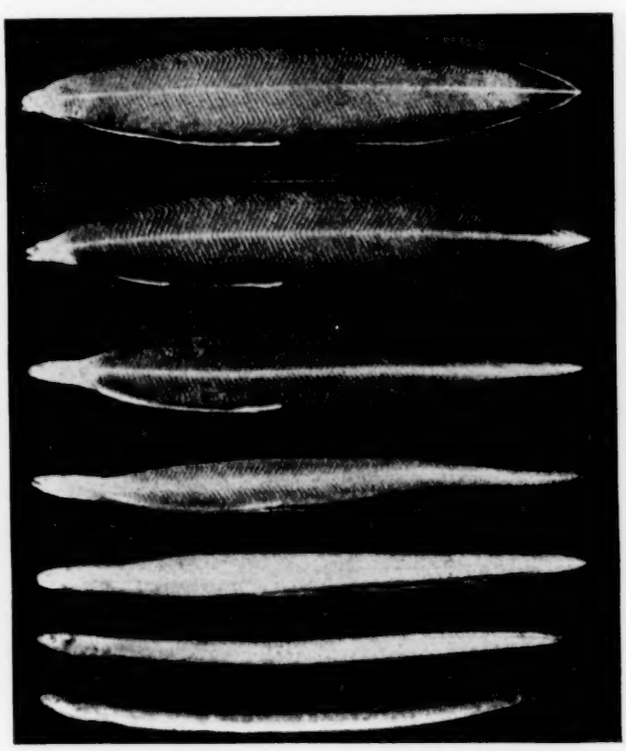

Fig. 1. Transition stages in the metamorphosis of the larva of the European eel, Anguilla vulgaris, from the Leptocephalis to the Elver stage. From Schmidt.

Grassi followed the transition from the flat transparent larval form through a series of stages into the young elver which has the general shape and appearance of the adult. In the transformation the larvae became less flat, more cylindrical and were actually reduced from 7.5 cm. to 6 or 6.5 cm. in length. Grassi inferred that the larval stages were deep sea forms driven to the surface by the currents in the straits of Messina, and that the real breeding places of the adult eels were the deep waters of the Mediterranean.

Grassi's discovery of the metamorphosis of the *Leptocephalis* furnished the key to the problem of the spawning migration of the eel,

though his deductions as to the spawning locality were wrong. He was working with larvae that had drifted in from the Atlantic.

The present state of the problem produced voluminous evidence, tending to locate the spawning grounds of the eel in mid-ocean to the north and east of the Bermudas and west of the Azores. The solution of the problem rests upon the correlation of the size, seasonal distribution and numbers obtained by collections in pelagic seining from numerous stations over the North Atlantic from the west coast of Europe across to North America, and from Labrador on the North to Porto Rico on the South. The collections were made by Danish expeditions under the direction of Schmidt, and by Hjort in coöperation with English scientific societies. Schmidt's surveys covered a series of years from 1906 to 1923. In 1905 the first larval specimen was taken off the west coast of Scotland and a second off the coast of Ireland. By 1908, 41 larvae had been taken at scattered stations. These varied in length from 4.5 to 8 cm., were nearly all from the North Atlantic about the Azores but a few as far west as Newfoundland. Specimens in process of metamorphosis were not found west of the European continental shelf. Furthermore, Schmidt's comparisons show that the smaller specimens were taken only at the most western stations. A summary of Schmidt's earlier collections indicated spawning in some North Atlantic area to the west of the Azores. Hjort's collections led to the same conclusions.

Schmidt by comparing the number of vertebrae in the larvae and Hjort by the number of larval myomeres were able to distinguish between the young of the European and American eels. An American collection made near the Bermudas and off the east coast of the United States was analyzed by Schmidt who found that 37 were specimens of the American eel and the remainder, all 4 to 5 cm. in length, of the European eel. This evidence confirmed his conclusion that there was a common spawning ground for the two species. In fact, he collected larvae of both species in the same net in the areas in question.

Schmidt in 1920 and 1921 following the leads of the earlier pelagic work made numerous seinings scattered widely over the North Atlantic. He now obtained literally thousands of larval eels. As he seined nearer the indicated spawning areas he obtained smaller and smaller larvae. In one haul on June 27, 1920, made at a depth of 50 feet, 778 larvae were collected which varied in length between 7 and 32 mm.

A statistical study of the variation in the sizes led Schmidt to classify the eel larvae in three groups which he called classes 0, I, II and III.

Class 0 was found in the great area bounded roughly by 50 and 70 west longitude and 20 and 30 north latitude, as shown in figure 4. The largest larvae were found only along the west coast of Europe undergoing metamorphosis into the elvers. The intermediate sizes I and II were distributed over the intermediate territory, growing ever larger toward the east. He concluded that these classes represented as many years of growth and that the elvers were three years old when they entered the fresh water streams of Europe.

By an analysis of the seinings it was shown that the collections contained American eel larvae intermingled with the European eel.

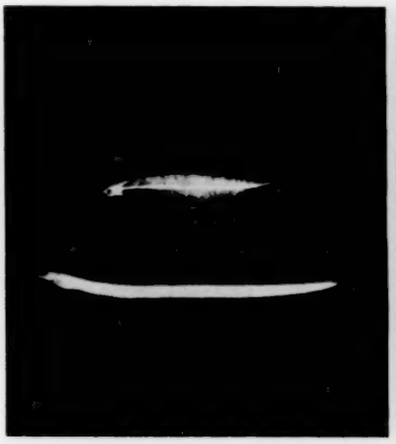

Fig. 2

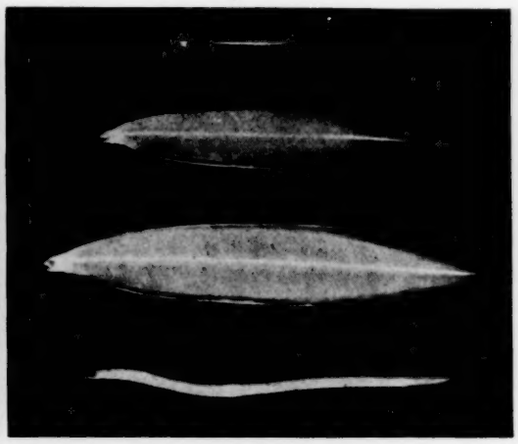

Fig. 3

Fig. 2. The larva and elvers of the American eel, Anguilla rostrata. From Schmidt.

Fig. 3. The stages O, I, II, III in the development of the larvae of the European eel, Anguilla vulgaris. From Schmidt.

But the American larvae were found in greater numbers somewhat further west and the center of their spawning area is to the west. Furthermore, the American eel passes through the metamorphosis into the elver in one year of larval development.

No pelagic eggs have been discovered of either of the two great species. However, Schmidt concludes that the spawning takes place in the region where the smallest larvae are the more common. Basing judgment on the fact that the small larvae rapidly increase in size as the summer months advance he infers that the spawning is in the early spring months. The whole story of the common eel is a brilliant example of a geographic statistical investigation in a complex problem not

open to solution by any known physiological methods for the study of individual reactions.

By deduction the story of the spawning migration of the eel can now be recapitulated as follows. The common eel, both American and European species, spend the adult life in feeding and growth in the fresh water. The mature eels migrate from the rivers to the sea, traveling southward for some distance, following a well-defined course along the

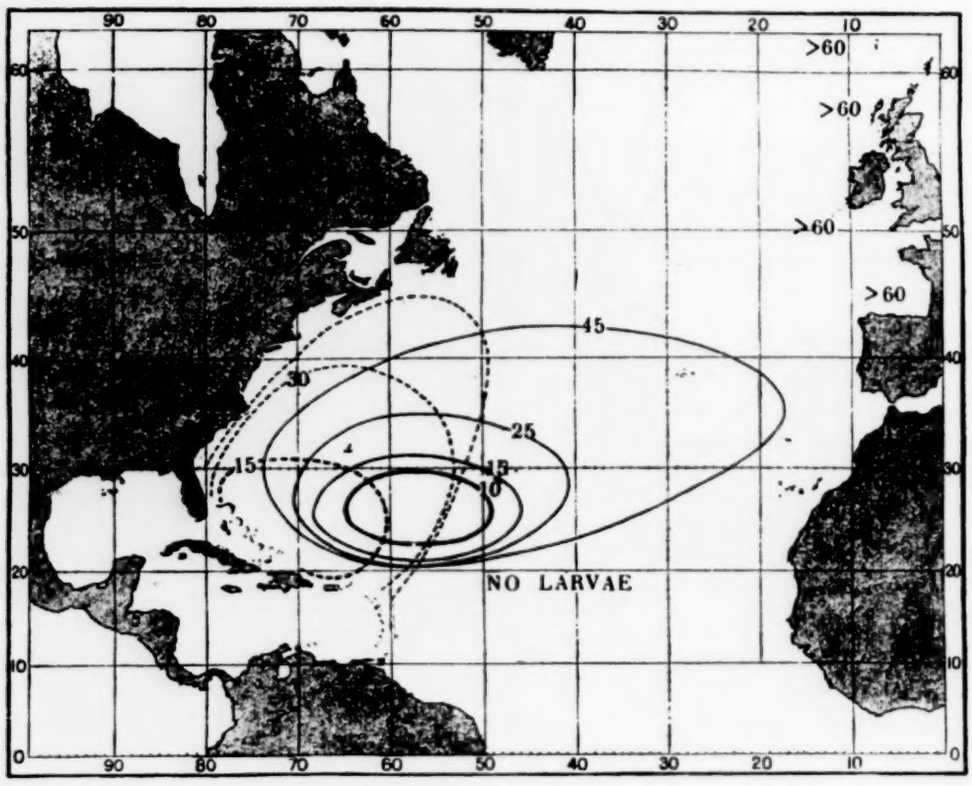

Fig. 4. The spawning areas of the European and American eels in the North Atlantic. The heavy solid curve represents the breeding area of the European and the heavy dotted curve of the American species. The lighter lines show the areas of dispersal and migration toward the respective coasts. From Schmidt.

continental shelf but ultimately disappearing into the deep waters of the North Atlantic. They spawn in the open ocean north and east of the Bermudas and Porto Rico, chiefly in longitude 50 to 70. The two species spawn in overlapping areas, the center for the European species somewhat to the east of that of the spawning area of the American species. We are left to infer by what magic the adult eels from two widely separated continents are led two to three thousand and more

miles across the ocean to the spawning area. As they are never afterward heard from it is inferred that they, like the salmon of the catadromous species, die after once spawning.

When the eggs incubated in the spawning area develop into larvae the two species migrate together following the great ocean currents to the northward and eastward. The larvae of the American eel with characteristic American acceleration metamorphose within one year and breaks away from the general northward migration to enter American rivers. After a three year trans-Atlantic voyage the European eels are transformed into elvers and promptly enter the European rivers. What physiological factors control the migration of the adults cannot at present be stated, although Schmidt has inferred that the migration is along the great ocean currents which flow to the westward near the equatorial limits of the North Atlantic. It is equally impossible to give a physiological basis for the long migration of the European larva from the spawning area to the European coast. Schmidt has also invoked the ocean current theory in explanation of this migration. That the larvae are carried northward with the Gulf Stream is easy to understand. However, at the elvers stage the migration is positive and aggressive, whatever that may mean in physiological terms, but why they aggressively leave the salt waters to enter the rivers is at present not explained.

THE SPAWNING MIGRATION OF ANADROMOUS FISHES. The anadromous fishes are those that begin life in fresh water but migrate to the sea for a greater or less period of feeding and development only to return to fresh water to spawn.

The great group of the salmon, the *Salmonidae*, already cited, furnishes the classical example of the anadromous spawning migration. Two genera are of especial interest. They are represented by *Salmo salar* and *Salmo gairdneri* of the North Atlantic and the North Pacific respectively, and by *Oncorhynchus tschawytscha*, the great king salmon of the Pacific coast of North America and Asia and its four related species,—*Oncorhynchus nerka*, the Alaska red salmon, *Oncorhynchus kisutch*, the silver salmon, *Oncorhynchus gorbuscha* the humpback, and *Oncorhynchus keta*, the dog salmon, all occupying an overlapping territory and of similar migrating and spawning habits. The species all have a variety of local names of Indian, Russian, Japanese or English origin. The last three species more often spawn in the coastal streams in the late fall after a short migration. The blue back

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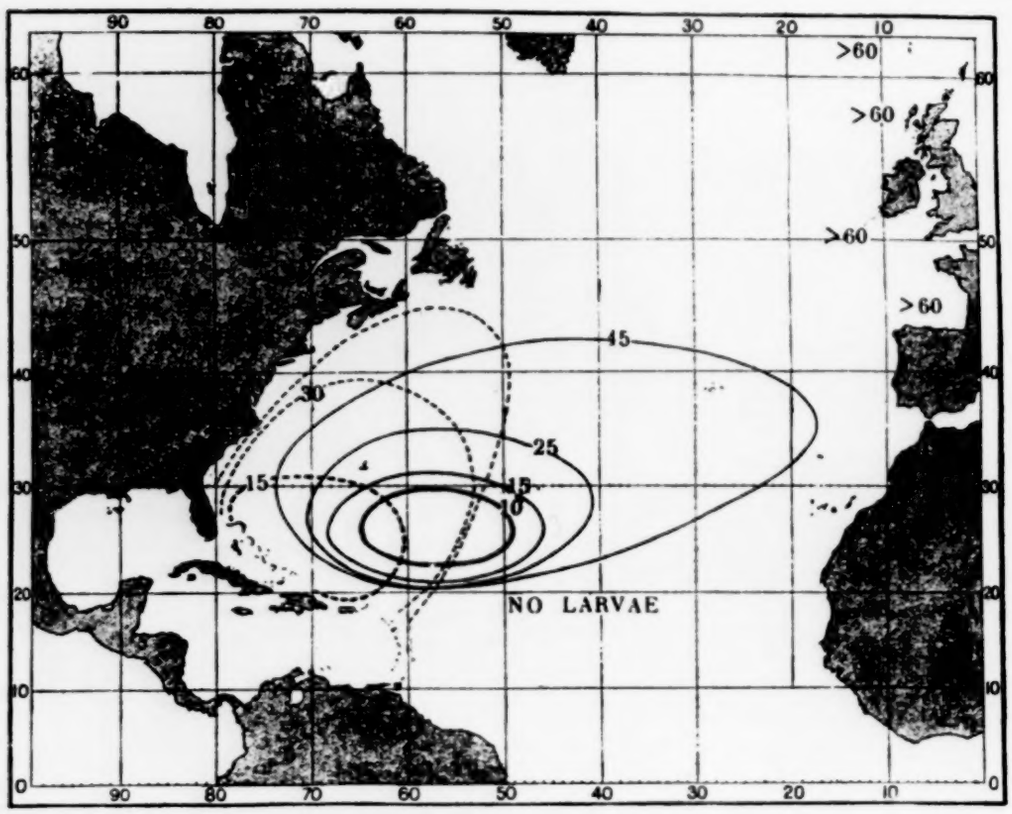

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(Columbia River strain), and redfish (Alaskan strain), of *O. nerka* ascend the rivers to their sources in mountain lakes where they spawn in the inlet brooks. The king salmon spawn in the headwaters of such rivers as the Sacramento, Columbia, Frazier and Yukon. The spawning grounds in such cases may be a distance of from a few hundred to over two thousand miles from the sea. The contrast between the representatives of the two genera of salmon is that the genus *Salmo* recuperates its exhausted condition after spawning by returning to the sea and feeding again. In the five species of *Oncorhynchus* the life history ends in death after the first spawning.

The Rhine salmon and the king salmon have been studied most carefully, not only with regard to their distribution, development, migration, spawning habits, but also with regard to the histological, chemical and physiological changes accompanying the different periods in the life history.

Friedrich Miescher, 1880 and 1897, published his classical studies on the salmon of the upper Rhine. Hoek, 1895, contributed a brief statistical survey of the salmon of the lower Rhine. These reports showed that the spawning migration up the Rhine occurred during the period from March to August. At the spawning grounds in the Alps, however, it was found that spawning was often delayed till November and December.

Miescher also noted that the sea salmon entering the river were fat and plump and in fine condition though the reproductive organs were comparatively undeveloped. The fish in the upper reaches of the Rhine at Basel, on the other hand, had lost weight and muscle, but the reproductive organs showed a relatively enormous gain in weight. Since he found that they did not eat during the journey Miescher was forced to conclude that the development of the ovaries especially, was by the transference of food material derived from the muscles. These deductions were based largely upon the changes in the chemical composition of the two tissues. Miescher believed that the Rhine salmon returned to the sea after spawning. Dahl, 1893 to 1895, proved the fact by marking 6708 spent salmon in the Norwegian rivers, afterward recapturing 54 in the sea and 112 on their return to fresh water. Gilbert has pointed out that only a small percentage of the Pacific species *Salmo Gairdneri* survive to return to the sea.

The Scottish salmon were studied intensively during the early nineties by Archer, Noel Paton, and Gulland and their associates, 1898. Particular attention was paid to the season and extent of the spawning migra-

tion, the distribution of salmon in the different Scottish rivers, the histological and chemical changes during the migration, the spawning period and the final recovery period. The data in the Report of the Fishery Board for Scotland were converted to equivalent weights of fish of standard length, which greatly facilitates comparison. Apparently the Scottish salmon come in from the sea during a prolonged season and remain in fresh water at the estuary for a period of acclimatization before ascending the rivers to the spawning grounds. At the latter spawning may be delayed for a period to permit further ripening of the gonads. The spawning fish showed a loss of weight of from 14 to 18 per cent. Archer further showed that males and females lost weight equally during the spawning migration.

Gulland, 1898, made histological studies of the alimentary canal describing an extensive desquamation or catarrhal change in the epithelium lining the stomach and intestines of the migrating salmon. No remains of digesting food were observed in the alimentary canals of these fish. The observations of Gulland have been questioned on the score of the technique used. However, the present writer has observed similar and more extensive degenerative changes in the Pacific salmon.

The digestive power of the stomach as measured by the peptic action of extracts of the gastric mucosa decreased rapidly in *Salmo salar*,— from 14.5 in March to 0.0 in July and August. The average acidity of the extract likewise decreased from 0.353 to 0.008 per cent. Recuperation took place later with a return of the peptic activity to 13.2 and the acidity to 0.190. A similar history applies to the Pacific species, *Salmo gairdneri*, which occasionally returns to the sea after spawning.

Miescher, 1880, considered the great lateral muscles the source of the food material consumed by the Rhine salmon in the ripening of the gonads. He observed fat droplets in the muscle and interpreted them as evidence of degenerative changes. He reported most marked degenerative changes in the superficial muscle plate along the side. This was the then unrecognized type of muscle later described in the king salmon by Greene, 1912, as a differentiated portion of the trunk muscle which possessed the power to store fats in unusual quantity.

Mahalanobis, 1898, made a detailed histological study of the muscle of the Scottish salmon. He confirmed the observation of fat droplets in the muscle saying, "In the fish this accumulation of fat sometimes reaches an enormous amount, and as a thin layer occurs under the sarcolemma." He considered this to be stored fat rather than evidence of degeneration saying, "Fatty degeneration is at present a misnomer.

The socalled degeneration is a mere fatty infiltration due to diminished utilization in the tissues." Later investigations have confirmed this view and conclusively shown that this is stored fat.

THE SPAWNING MIGRATION OF THE KING SALMON. The king salmon of the Pacific, *Oncorhynchus tschawytscha*, is the largest and most prized member of its genus. It often reaches a length of over one meter and while the average weight is from 8 to 10 Kilos, individuals may weigh as much as 35 to 45 Kilos. The biological history of the salmon and its commercial exploitation has attracted a great deal of scientific investigation as well as governmental and private attention and interest. There are a number of features in the history of the king salmon that have been difficult to determine and that have proven unique in physiological interest as the life story has unfolded.

The king salmon was given scientific description by the Russian, Krascheninnikow, 1768. Its present name and description is accredited to Walbaum. This species spawns in most of the rivers of the north Pacific coast, from the Sacramento River in California on the south, to British Columbia, Alaska and to Kamchatka on the Asiatic coast. The growing adults are found in the ocean along this wide extent, but whether their ocean life is spent near the shore or in mid-ocean is not yet conclusively determined. Its general biological history has been contributed by numerous investigators. Among these may be found Stone, Jordan, Evermann, Gilbert, Schofield, Rutter and Rich.

Evermann, 1896, in investigations of the king salmon and the red fish in the Columbia River basin observed that the king salmon all die after the single spawning act. This was fully confirmed by Rutter and by all later investigators. Each of the five species of *Oncorhynchus* is now known to die after spawning.

Rutter, during five years of field study of the king salmon, repeated Dahl's experiment on this species by marking a number after they were artificially spawned and followed their career after they were returned to the streams. All died.

Rutter also summarized the evidence that the king salmon, like the Atlantic *Salmo salar*, does not take food during the spawning migration; apparently the salmon cease to feed while still in the ocean, or at the time they enter the brackish water at the mouths of the rivers. This abstenance from food is of the greatest significance in view of the long period in fresh water, and the tremendous amount of energy required to stem the currents of the mountain streams in the journey to the cold waters of the spawning grounds.

The time and the rate of speed of migration in the fresh water journey has been deduced from the records of the commercial fisheries and from direct scientific experiments.

Rutter, in 1901, weighed and numbered one hundred and fifty adult salmon and returned them to the river at Rio Vista in a pioneer experiment on the Sacramento River. Of these he recovered three as shown in the data of the table below. These traveled at an average rate of from four to five miles a day and lost from 15 to 26 per cent in weight.

In 1908 Greene marked and liberated fifty-nine king salmon, silver salmon and steelheads within the estuary of the Columbia River. Numbered aluminum buttons were used. Seventeen of these fish were recovered at various stations of the river from near the point of marking to the Seufert fisheries 210 miles up stream. The king salmon were all recaptured near the point of marking, four of the silver salmon traveled 210 miles at a maximum average rate of 7.5 miles a day. This was a

TABLE 1

Salmon migration rate experiment at Rio Vista on the Sacramento River above tide water—Rutter

NUMBER AND SEX	DATE RELEASED	CAPTURED AGAIN	PLACE RECAPTURED	TIME OF JOURNEY	WEIGHT WHEN RELEASED	WEIGHT WHEN CAPTURED	PER CENT OF LOSS
				days	*grams*	*grams*	
8 ♀	Sept. 20	Nov. 23	Mill Creek	64	13,930	10,180	26
91 ♀	Sept. 24	Nov. 20	Mill Creek	56	8,470	7,160	15
43 ♂	Sept. 20	Nov. 25	Battle Creek	66	10,080	6,275	25

comparatively lower speed than that obtained from the data of commercial catches from rapidly migrating schools of spring salmon.

Gilbert and Rich, 1922, 1923, have marked Alaska salmon on a large scale, adopting the system of individual numbering. They have shown that the salmon migrate at a much higher speed than formerly assumed. In 1922 they tested for the first time the average speed along the migratory sea routes of the Alaskan coast and found the rate as high as eighteen to twenty miles per day. They secured data on the migration of all the species of *Oncorhynchus*. The culmination of this brilliant experimental field work by this method was in the marking of about 10,000 adult salmon as they first appeared in the schools off the islands and coast of the Aleutian peninsula. Of this number 3296 were recaptured.

Probably the longest migration of salmon in fresh water is on the Yukon River of Alaska. The spawning migration begins promptly as the ice goes out of the river in June and the salmon make a direct and

uninterrupted run to unknown spawning beds in the headwaters. Data by Gilbert on the migration rate during the year 1920 are given in table 2.

The king salmon covered the 1500 miles to Dawson at the rate of fifty-two miles per day. Since the salmon swim against the swift currents of the Yukon, the actual output of energy is much greater than the mileage indicates.

TABLE 2

First dates of capture of migrating king salmon at a series of localities along the Yukon River during the season of 1920, from C. H. Gilbert

LOCALITY	DATE	APPROXIMATE NUMBER OF MILES TRAVELED	MILES TRAVELED PER DAY
South mouth of river	June 13		
Run begins south mouth	June 15		
Pilot Station	June 20	107	21
Marshall	June 20	144	29
Russian Mission	June 21	204	34
Paimiut	June 22	259	37
Holy Cross	June 23	279	35
Halls Rapids, above Anvic	June 24	346	38
Camp 51 miles below Kaltag	June 27	440	37
Kaltag	June 28	491	38
Koyukuk	June 29	555	40
Whiskey Creek, above Louden	June 27	622	52
Ruby	June 27	659	55
Tanana	June 28	804	62
Fish Creek above Rampart Rapids	July 3	851	47
Circle	July 11	1,227	47
Charlie Creek	July 12	1,317	49
Eagle	July 13	1,402	50
DeWolf's Fish Camp	July 14	1,478	51
Dawson	July 14	1,504	52

During the last three decades the method of interpreting age by the growth rings and markings of the scales, introduced by the early work of Dahl and of Johnson, has been applied to the Pacific salmon by various investigators. To Gilbert, however, must be given the credit for perfecting the method to its present high degree of accuracy in interpretation. As now understood, the history of the salmon in fresh water and in the sea is indelibly marked on its scales by the distribution and character of the growth rings.

By coupling the method of studying the migration of individually

marked salmon with a study of the history of that salmon as written on its scales, Gilbert has immensely extended the biological story of the five species of *Oncorhynchus*. We can not go into details here, but for purposes of understanding the spawning migration, certain of these facts and conclusions are stated: 1. From the countless millions of feeding salmon, those that have reached certain limits of maturity, separate out and begin the migration journey. The age varies with the different species. In the king salmon it averages 4 years but varies from 3 to 7 years, the longer life characterizing the schools of the colder waters such as the Yukon. In the Alaska red salmon the average age is 4 years, and in the humpback 3 years. 2. The salmon migration takes a prompt and comparatively direct sea route from the feeding areas of the ocean to the rivers of their spawning grounds. 3. The evidence becomes ever more and more conclusive that the spawning occurs in the identical streams in which the fish as young were reared. The salmon from different stream sources co-mingle in the great migratory schools off the Alaskan Peninsula. They separate each going to its natal stream as the marine migration terminates in the fresh water lap of the journey.

We owe the final and confirmatory chapters of the story of the seaward migration of the young king salmon to the experiments of Scofield, of Rutter and of Rich. Rutter, 1902, made exhaustive surveys of stream populations of young fry and parr in the Sacramento basin, studying the down river progress by the method of daily seining and counting the variation in numbers and sizes and by following the migration of individually marked parr. Rich exhaustively studied the seaward migration of young king salmon by combining the scale history and the locality—size methods. His graph showing growth of the young in the Columbia River is given below. In the Sacramento River the corresponding sizes and ages occur earlier in the season but are similar in type.

Anadromous fishes must meet the physiological necessity of adapting the body to the transition from fresh water to sea water, and the reverse. Fresh waters of the mountains streams in which the young are hatched have little or no saline content, while the inorganic content of the sea water may depress the freezing point from 2 to 3.5°C. The sea water of Monterey Bay has a Δ of -1.924°C.

Mosso, 1890, demonstrated that the osmotic pressure of the body fluids of the cartilaginous fishes, such as *Scyllium*, is that of the sea water in which they live. Botazzi, in 1897, found that the osmotic

pressure of the body fluids of a series of marine invertebrates was that of their environment. Greene, 1904, made similar observation regarding the California Hagfish, *Bdellostoma Stouti*. The skin of the latter is completely permeable and if the fish is transferred from sea water to fresh water it will die in a few minutes with the development of water rigor. Garrey found that the skin of other fish, such as *Fundulus*, was impermeable and that these forms could safely be transferred from sea water to fresh water and back again.

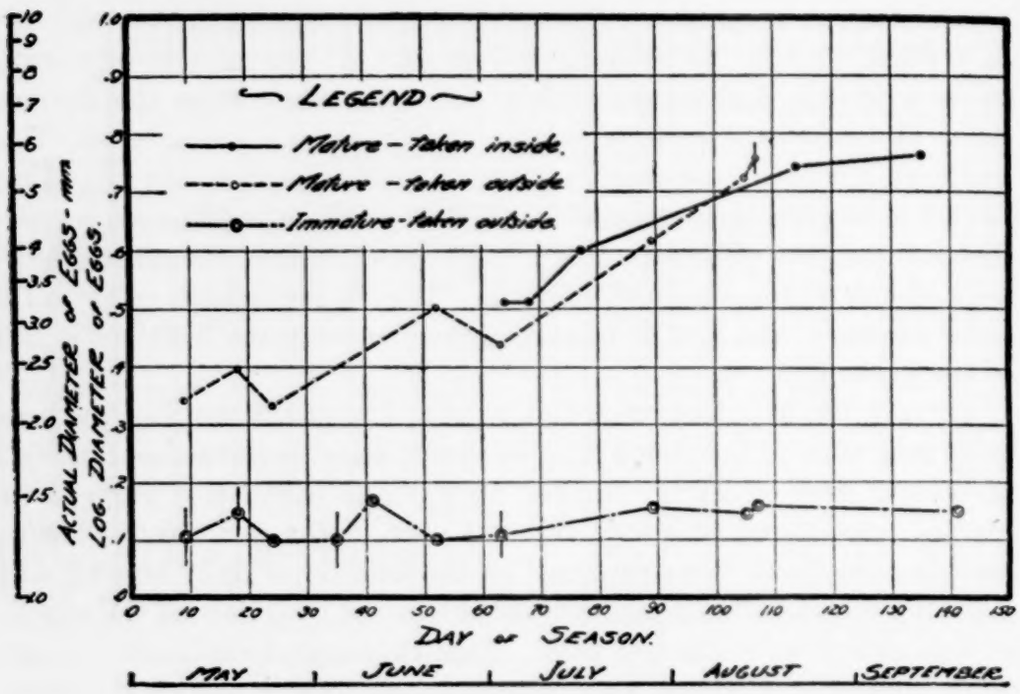

Fig. 5. The growth of the ovaries of maturing king salmon compared by the diameters of the developing eggs from fish within the mouth of the Columbia River and from the ocean outside. The figure shows the rapid increase in size during the fourth or spawning season. Little growth in ovary is shown by the younger fish, the bottom line in the graph. From Rich.

The skin of the salmon is relatively impermeable to inorganic salts. It neither absorbs the salts from the sea water nor imbibes water while the fish is in fresh water streams. Greene, 1904, found evidence of some blood dilution during the transition to fresh water. The average depression of the freezing point of the blood from 18 salmon collected from salt water, Monterey Bay, was −0.762°C., the blood of salmon from the head of tide water on the lower Sacramento River averaged −0.737°C., while the mean of the blood of 24 salmon from the McCloud

River spawning grounds was −0.668°C. The depression of the freezing point in this series was decreased about 12 per cent. The permeability of the salmon skin in fresh water may be somewhat increased under pathological conditions, by injury to the expidermis from the growth of infecting bacteria, molds etc., or from lesions from fighting or other accidental injuries. A careful selection of specimens without evidence of skin lesions would doubtless decrease the observed degree of blood dilution.

The young salmon acquire an impermeability to sea water at a late stage in their development, and do not go out to sea until they attain the fingerling or more mature stage of growth. Rutter, 1902, found that salmon eggs and younger fish could not survive the transfer to sea water. Not until the young are sixty days old or more will they withstand sea water.

Commercial fishermen seining at the mouth of the Columbia River recognize that schools of salmon may advance and drop backward with the ebb and flow of the tides. Marked salmon also were recaptured at down river points on the Columbia from six to fourteen days after liberation. These observations further emphasize the importance of the acclimatization period in the migration.

Evidently there is a definite period of acclimatization, both when adult salmon and when young make the transition from fresh water to sea water, or the reverse. During this period the fish spend days or even weeks in brackish water, swimming back and forth with the ebb and flow of the tides until the transitional adjustments are completed.

CHANGES IN THE CHEMICAL COMPOSITION OF THE KING SALMON DURING THE SPAWNING MIGRATION. Chemical studies on the king salmon were reported by Greene, 1915 and 1919. Preliminary studies were made on fish from the Sacramento River basin, from stations at Monterey Bay on actively feeding marine adults, from the head of the tide water at Black Diamond, and from the spawning fish at Baird on the McCloud River in Northern California. More complete data were later obtained from fish taken in the Columbia River basin. The stations along the latter were located: 1. At Ilwaco, Oregon, at the mouth of the Columbia. 2. At Warrendale, Oregon, 130 miles from the sea. 3. At Seufert, Oregon, 210 miles from the sea. 4. At Ontario, Oregon, on the Snake River, 700 miles from tide water. At the spawning grounds at Cazadero, Oregon, located on a branch of the Willamette, 130 miles from sea.

The changes in the composition of the muscle are given in tables 3 and 4.

Miescher and Noel Paton and his associates, both emphasized the changes in the amount and composition of the muscle of the *Salmo salar* during the spawning migration. The changes, however, lack the dramatic character of those observed in *Oncorhynchus tschawytscha*.

In the king salmon the Sacramento series reveals a loss of over one-half of the total muscle, 51.6 per cent, by the time spawning is completed. Not only is there a reduction in the total mass of the muscle but there is also an equally striking change in the composition of the remaining portion. This comprises a diminution in the total solids with a corresponding increase in the percentage of water. The fat which at the beginning of migration was 18 per cent of the standard weight of muscle was reduced to 1.6 per cent at spawning. The total solids of muscle were reduced from 25.9 per cent of the total weight of the standard fish to 6.9 per cent calculated weight of the standard weight of the spawning fish. These data also revealed the fact that the salmon that had just passed through tide water was 5.6 per cent heavier in total weight of muscle, but 1.3 per cent lighter in weight of dry constituents of muscle than the standard fish. These last changes are part and parcel of the acclimatization process in coming out of the salt water.

TABLE 3

A comparison of average food values of Sacramento salmon collected from the sea, the head of tide water, and from the spawning grounds on the McCloud River, California, reduced to uniform standard length of 860 millimeters, from Greene, 1915.

				WET MUSCLE					DRY MUSCLE		
	Standard length	Total weight	Standard weight	Weight of wet muscle	Standard weight of wet muscle	Per cent of muscle in standard fish	Protein per cent in muscle	Fat per cent in muscle	Weight of dry muscle	Per cent of standard weight of dry muscle	Per cent of dry muscle in standard fish
	mm.	*grams*	*per cent*	*grams*	*per cent*						
Sea	860	10,080	100	6,836	100	67.8	15.6	18.0	2,616	100	25.9
Tide water	860	11,177	110	7,220	105.6	71.6	16.9	14.6	2,581	98.7	25.6
Spawning grounds	860	7,531	74	3,311	48.4	32.8	14.4	1.6	682	26.1	6.8

These percentages of table 3 cover two factors of loss, one the gross disintegration of muscle tissue, and the other, the decrease in percentage of protein per unit of the protoplasm that remains. The latter is the factor on which the argument for deposit protein or reserve protein is based. The first factor, of protoplasmic disintegration, is a protein resource of the salmon in migration.

The muscle of the salmon collected at the estuary of the Columbia averaged 15.5 per cent in neutral fat, table 5. This series was collected late in the season (August) and contained less fat than observed in salmon entering earlier in the year. The percentage of fat was more often above 20 for the composite muscle sample. The amount of fat is much greater, amounting to 30 per cent or more, in the superficial division of the great lateral muscle. This is the dark type of muscle and has a greater capacity for storing fat. At the spawning grounds the muscle fats have almost disappeared. The average shown in the table is 2.2 per cent, but the lowest observed was only 0.7 per cent.

Fat storage in the salmon occurs principally in the muscles and the inter-muscular connective tissues. Histological studies, page 228, show that this storage takes place not only "among the muscle fibers but also within certain types of muscle fibers." This stored fat represents material super-added upon the active muscular tissue and should be eliminated from consideration in studying changes in the active protoplasm.

Therefore, computations have been made on the basis of the fat-free weight of muscle. Data calculated on this basis represent most closely the chemical changes in the active living tissue.

The proteins of salmon muscle from the three lower stations on the Columbia River are very constant, 20.07, 20.19 and 20.09 per cent, but after a seven-hundred-mile journey to Ontario the protein percentage decreases to 18.2, and at spawning it is only 14.02 per cent. These figures prove an astounding loss, 30 per cent of the initial tissue protein at the beginning of the migration. These data have been interpreted by the author as supporting a hypothesis of protein storage in the salmon muscle cells.

The carbohydrates were not analyzed in the series represented in table 4. However, in a series of analyses from the Sacramento River basin glucose was shown to be present in very minute amounts in feeding salmon but absent during the fasting migration. In view of our present theories of fat metabolism, the dearth of carbohydrates in the tissues of the migrating salmon must be emphasized. The muscles of

TABLE 4

Composition of the muscle tissue of the king salmon, Oncorhynchus tschawytscha, during the spawning migration. Greene, 1919*

COLLECTING STATIONS	DATE	SALMON NUMBER AND SEX	WATER		SOLIDS		PROTEIN			PHOSPHORIZED FATS			NEUTRAL FAT		ORGANIC EXTRACTIVES			ASH		
			Wet	Fat-free	Wet	Fat-free	Wet	Dry	Fat-free	Wet	Dry	Fat-free	Wet	Dry	Wet	Dry	Fat-free	Wet	Dry	Fat-free
	1908		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Ilwaco, Wash. Tidewater	Aug. 15	1276♂	62.5	74.5	37.46	25.30	16.92	45.16	20.16	0.76	2.56	1.14	16.1	42.9	2.62	6.99	3.12	0.89	2.36	1.05
	Aug. 18	1279♀	63.8	74.6	36.23	24.88	17.25	47.60	20.18	1.03	2.84	1.21	14.5	40.1	2.35	6.55	2.75	1.06	2.92	1.24
	Aug. 20	1281♀	63.2	75.2	36.80	24.78	16.73	45.46	19.88	1.00	2.71	1.18	15.8	43.0	2.48	6.74	2.95	0.73	2.07	0.91
Average			63.2	74.8	36.83	24.99	16.97	46.07	20.07	0.93	2.70	1.18	15.5	42.0	2.48	6.76	2.94	0.90	2.45	1.07
Warrendale, Ore. 130 miles from the sea	Aug. 5	1259♂	60.3	75.4	39.66	24.58	16.32	41.15	20.40	0.77	1.94	0.96	20.0	51.2	1.80	4.55	2.25	0.78	1.94	0.98
	Aug. 5	1261♀	63.4	74.3	36.57	25.59	17.11	46.80	20.04	0.60	1.62	0.70	15.1	39.9	3.16	8.65	3.71	0.94	2.56	1.10
		1262♂	62.2	75.8	37.76	24.21	16.15	42.79	19.67	0.75	1.99	0.92	17.9	47.3	2.15	5.71	2.62	0.82	2.17	1.00
	Aug. 7	1263♀	64.7	74.4	35.28	25.58	17.96	50.90	20.65	0.57	1.62	0.66	13.0	36.9	2.84	9.04	3.26	1.03	2.93	1.19
Average			62.7	75.0	37.32	24.99	16.88	45.41	20.19	0.67	1.79	0.81	16.5	43.9	2.49	6.99	2.96	0.89	2.40	1.07
Seufert, Ore. 210 miles from the sea	July 11	1040♂	64.5	75.2	35.46	24.79	17.39	49.05	20.27	0.95	2.67	1.10	14.2	40.0	2.02	5.71	2.36	0.91	2.56	1.06
	July 12	1056♀	62.9	74.5	37.10	25.47	16.88	45.49	20.00	0.80	2.16	0.95	15.6	42.0	2.89	7.79	3.43	0.93	2.51	1.10
	July 13	1060♀	63.2	76.4	36.83	23.58	16.27	44.19	19.65	—	—	1.06	18.2	49.5	1.57	4.26	1.90	0.77	2.09	0.93
	July 15	1074♂	60.3	74.9	39.67	25.12	16.42	41.29	20.38	0.87	2.19	1.09	19.4	48.9	2.04	5.13	2.53	0.89	2.23	1.10
	July 23	1179♂	63.4	74.0	36.56	26.01	17.60	48.13	20.52	0.78	2.14	0.91	14.2	39.0	2.92	7.98	3.40	1.01	2.76	1.18
	July 31	1180♀	65.8	74.2	34.21	25.83	17.49	51.13	19.72	0.75	2.19	0.85	11.3	33.0	3.47	10.14	3.91	1.20	3.51	1.35
Average			63.4	74.9	36.64	25.15	17.01	46.55	20.09	0.83	2.26	0.96	15.5	43.2	2.49	6.84	2.92	0.95	2.61	1.12

Ontario, Ore. 700 miles from the sea	Sept. 6	1312 ♀	70.4	76.2	29.62	23.81	17.36	58.61	18.79	0.63	2.11	0.67	7.3	24.7	3.14	10.61	3.40	1.16	3.90	1.25
	Sept. 7	1314 ♂	68.8	77.0	31.22	23.03	16.19	51.85	18.11	0.52	1.66	0.73	10.8	24.5	2.69	8.63	3.01	1.05	3.35	1.17
	Sept. 7	1315 ♀	68.2	77.2	31.82	22.81	15.87	49.88	17.96	0.14	0.45	0.16	11.7	36.6	3.21	10.10	3.62	0.98	3.08	1.10
	Sept. 8	1316 ♂	67.4	76.5	32.60	23.46	16.50	50.54	18.71	0.20	0.60	0.22	11.9	36.6	3.00	9.21	3.41	1.07	3.28	1.21
	Sept. 7	1317 ♂	71.2	76.7	28.82	23.24	17.33	60.14	18.69	0.28	0.98	0.30	7.3	25.2	2.96	10.26	3.18	0.99	3.43	1.07
	Sept. 9	1321 ♀	69.0	77.8	30.98	22.20	15.90	51.31	17.91	0.37	1.20	0.42	11.3	36.4	2.52	8.15	2.84	0.90	2.92	1.00
	Sept. 11	1326 ♀	67.9	77.8	32.11	22.19	15.05	46.87	17.25	0.19	0.60	0.22	12.7	39.7	3.07	9.56	3.52	0.87	2.70	0.99
Average			69.0	77.0	31.02	22.96	16.31	52.74	18.20	0.33	1.08	0.39	10.4	33.4	2.94	9.50	3.29	1.00	3.24	1.11
Cazadero, Ore. The spawning grounds†	Aug. 25	1294 ♀	80.2	80.8	19.77	19.20	14.48	73.17	14.58	0.45	2.26	0.45	0.7	3.5	3.08	15.57	3.10	1.06	5.37	1.07
	Aug. 25	1295 ♂	78.1	80.5	21.89	19.51	14.41	65.83	14.85	0.44	2.01	0.45	2.9	13.5	3.17	14.50	3.27	0.91	4.17	0.94
	Aug. 26	1296 ♀	78.8	81.8	21.21	18.18	13.37	63.05	13.88	0.18	0.84	0.18	3.7	17.4	3.00	14.17	3.12	0.96	4.51	0.99
	Aug. 26	1297 ♀	81.4	83.0	19.56	17.06	12.82	69.09	13.05	0.29	1.56	0.29	1.8	10.2	2.71	14.63	2.76	0.87	4.78	0.90
	Aug. 27	1298 ♂	81.0	82.2	18.97	17.79	13.62	71.79	13.82	0.45	2.35	0.45	1.4	7.6	2.69	14.17	2.73	0.98	5.19	1.00
	Aug. 27	1299 ♀	79.3	81.8	20.68	18.15	12.85	62.16	13.26	0.62	3.02	0.64	3.1	14.9	3.22	15.58	3.32	0.90	4.34	0.93
	Aug. 28	1300 ♂	78.8	80.6	21.19	19.44	14.41	67.99	14.73	0.60	2.86	0.62	2.2	10.3	3.09	14.57	3.16	0.91	4.29	0.93
Average			79.7	81.5	20.32	18.48	13.71	67.58	14.02	0.43	2.13	0.44	2.2	11.1	2.99	14.74	3.07	0.94	4.66	0.97

* The figures are averages of duplicate samples, with the single exception of no. 1294. The computations are made on three bases,—fresh wet sample as taken, the dry sample, and the fat-free sample.

† The spawning salmon have been in fresh water without eating for a time estimated as 4 or 5 months.

feeding salmon from Monterey Bay contain a small amount only, from 14 to 15 mgm. per cent of glycogen, while those of Bolinus Bay, probably fasting, contain only traces. The muscle from specimens from the spawning grounds contains no glucose.

The liver of feeding salmon averaged only 380 mgm. per cent, and the spawning salmon of the McCloud River 52 mgm. per cent of liver glycogen. The glucose of the ovary is a trifle higher. It is quite apparent that the salmon possesses some other method of burning fats than "in the flame of the carbohydrates."

The organic extractives in the muscle increase slightly, from 2.94 to 3.07 per cent. Carl H. Greene, 1919, found that the non-protein amino nitrogen increased slightly at the beginning of the migration and then remained constant in relation to the water of the tissue. Since the increase was very marked in proportion to the decreasing protein, he drew the conclusion that, "Evidently the amino-acids are liberated at the expense of an ever increasing wastage of the muscle substance;" and that the tissue water volume is the regulating factor and not the proteins from which they are derived. He did not find any essential changes in the creatine content during the migration, although there was a sharp increase in ratio to the muscle proteins as the period was prolonged.

TABLE 5

Changes in the extractive fractions of muscle of salmon during migration. C. H. Greene

STATION	LOCATION	PROTEIN RESIDUE	WATER EXTRACT	EXTRACTIVE NITROGEN	AMINO NITROGEN	TOTAL CREATINE NITROGEN
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
Ilwaco.....	Tide-water	20.3	4.79	0.482	0.061	0.081
Warrendale.....	130 miles	19.9	4.65	0.524	0.076	0.094
Seufert.....	210 miles	19.7	4.70	0.521	0.078	0.078
Ontario.....	700 miles	18.3	4.75	0.553	0.074	0.089
Cazadero.....	Spawning grounds	13.8	4.34	0.534	0.084	0.089

The inorganic constituents of muscle remain quite constant, 1.07 to 0.97 per cent of the fat-free tissue. These figures further suggest the independence of the salmon of the inorganic constituents of the water in which it lives.

GROWTH AND DEVELOPMENT OF THE REPRODUCTIVE ORGANS DURING THE SPAWNING MIGRATION. The development and maturing of the

reproductive gonads of fishes in relation to the cycle of body growth is best known from data obtained from the salmon. Miescher, 1880, was the first to emphasize the fact that the ovaries of the Rhine salmon made large growth after the fish entered fresh water. He proved that the gonads make 97 per cent of the growth from March to November and while in the upper river. The ovaries of salmon at Basel in March weighed 0.77 per cent of the body weight. The relative weight increased rapidly through the spring and summer months and very rapidly in the autumn until at the spawning time in November it was 26.72 per cent of the body weight. The male gonads also grow to maturity during the fasting sojourn in fresh water, from 0.105 per cent of the body weight in March to 6.10 per cent in October.

The spawning time of the Rhine salmon extends from November 15 to December 15, which represents an active growth period of the ovaries and testes during eight months of fasting. Since corresponding seasonal differences in the ovaries of salmon from the mouth of the Rhine were reported by Hoek, we must point out that this profound growth which occurs in the spawning year is not because of the sojourn in fresh water, but that it is a phase of the development cycle and produced by the factors that induce sexual maturity at an approximate stage in growth.

Noel Paton and Dunlap, 1898, compared the size of the ovaries and testes of large numbers of salmon from the estuaries and from the upper waters of the chief salmon rivers of Scotland. Their statistics for the summer and autumn months are assembled in table 6.

The statistics of the above table show that salmon are entering from the sea during a long season. The reproductive organs are already growing rapidly when the late arrivals enter the rivers. These data we interpret as a confirmation of the deduction from the data of Miescher, i.e., that growth of mass of reproductive organs is stimulated by other factors than the influences incident to the life in fresh water. In light of present advances in physiology it would be more logical to assume an internal chemical stimulation, or hormone.

RATE OF GROWTH OF THE OVARIES OF THE KING SALMON. During the migration of the king salmon the growth of the reproductive gonads, like that of the Rhine salmon, is rapid during the months immediately preceding the spawning. In the Columbia River the increase in the average weight of the ovaries during the season of 1908 was from 500 grams at the estuary, in salmon entering in August, to well over 2000 grams on the spawning beds in September in salmon that had left the sea during the spring months. It is well known that the size of the

ovaries of salmon entering the Columbia River during the fall months is very much greater than in the migrants of the spring months. Apparently a period of rapid growth of the reproductive organs in the king salmon occurs during the season of their spawning migration, whether it begins in the sea or in the fresh water.

A statistical study by Rich of the size of the ova in the sea-run of king salmon of the different year classes showed a progressive but slow growth of the diameters of the ova through the first, second and third years of the salmon's sea life. During the third year there was a more

TABLE 6

A comparison for the spring, summer and fall of spawning season, of average weights of the reproductive gonads of migrating salmon in Scottish rivers. Certain recalculations are added to the data from Noel Paton and Dunlap, 1898

SEASON	WEIGHT OF STANDARD FISH (FEMALES ONLY)		MUSCLES OF STANDARD FISH		OVARIES OF STANDARD FISH IN GRAMS AND PER CENT OF BODY WEIGHT		TESTES OF STANDARD FISH IN GRAMS AND PER CENT OF BODY WEIGHT	
	Estuary	Upper river	Estuary	Upper river	Estuary	Upper river	Estuary	Upper river
	gms.	*gms.*	*gms.*	*gms.*				
1. May and June	10,185	9139	6386	5839	121–1.2%	263–2.9%	13.3–0.15%	43.2–0.44%
2. July and Aug.	10,781	9616	6901	5887	284–2.7%	510–5.2%	41.2–0.38%	115.2–1.33%
3. Sept. and Oct.	11,648	9646	6055	4116	1439–12.4%	2230–23.2%	260.0–2.71%	269.0–3.32%

rapid growth shown in the logarithmic growth curves, one of which is presented in figure 5.

Salmon from the spring run of the Columbia enter the Willamette River in April and May. At that time their reproductive organs are relatively small. These salmon spawn in September. The ova in the meantime have grown to weights of from 1617 to 2243 grams, or approximately 25 per cent of the body weight. Such excessive growth in the absence of any food intake is phenomenal and will be discussed later.

THE CHEMICAL COMPOSITION OF THE OVARIES OF THE KING SALMON. The ovaries of the king salmon were analyzed quantitatively by Greene,

1921. His data are presented in full for 15 fish chosen from the five stations on the Columbia River, table 7. Although the ovaries are growing rapidly in mass it will be noticed that the chemical groups are maintained in a relatively constant ratio throughout the migration. The quantity of each constituent group increases slightly in the early stages of the journey only to fall off as the maturity of the ovary and ripening of ova are completed.

The percentage of fat is greater during the intermediate stages of the journey and at the time the body fats are in greater flux. The average is definitely less at the time of spawning. The average figures are 14, 13.5, 17.5, 13.6 and 10.6 per cent for the five successive stations. In two specimens of freshly spawned ova the percentages were 11.7 and 9.2 respectively. Computed on the basis of percentages of water-free material the differences are accentuated as shown by the data in table 7. On this basis of comparison there is 30 per cent of neutral fat at the beginning of the journey, 36 per cent in the intermediate stages and 24 per cent (28 to 22 per cent) in the ovary of the spawning salmon.

The average phospholipin content of the ovary was 4.7 per cent at the beginning of the journey. This percentage was progressively reduced as the ova matured in the ovary. In the free eggs at the spawning the average was only 2.8 per cent. The compound fats are never so great in the king salmon ovary as in the hen's egg.

The ovary of the king salmon contains an unexpectedly high per cent of protein in all stages of development. The total averages about 30 per cent of the fat-free samples at all stages. The figures are 29.82, 30.02, 31.07, 31.33 and 29.29 per cent.

The living protoplasm represents a very negligible percentage of the total ovary, or of the ripe eggs. The proteins, fats and lecithins are foods laid down in the yolk as extraneous deposits for the growth and development of the fertilized embryo. The unique fact is that the high proportion of protein is maintained at so constant a level during the entire migration. There is both mass exhaustion and a percentage depletion of the sources of proteins in the great muscles, as shown in earlier discussion of the chemistry of the muscles. The mechanism of transfer of proteins is adequate to maintain a constant ratio of deposit.

Miescher reported only 15.6 per cent of protein in the ovary of the Rhine salmon. Noel Paton found an increase in albumen during the migration to a maximum of 25 per cent ($N \times 6.25$). Nelson and Greene, 1921, obtained an average of only 25.8 per cent of protein in the ovaries of the fresh water gar, Lepidosteus.

TABLE 7

Showing the length, weight and date of collection, the ovarian weight, and the groups of chemical constituents of salmon ovaries collected from five stations, representing stages of the spawning migration, Columbia River, season of 1908. Greene, 1921

COLLECTING STATIONS	DATE	FISH NUMBER	LENGTH, TIP TO TIP	WEIGHT	WEIGHT OF OVARIES	SAMPLE NUMBER	WATER		SOLIDS		PROTEINS			PHOSPHOLIPINS			NEUTRAL FATS		ORGANIC EXTRACTIVES			ASH		
							Total	Fat-free	Total	Fat-free	Total	Water-free	Fat-free	Sample	Water-free	Fat-free	Sample	Water-free	Sample	Water-free	Fat-free	Sample	Water-free	Fat-free
	1908		*mm.*	*grams*	*grams*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Ilwaco, Wash., Estuary	Aug. 18	1,279	870	11,215	501	1,298	54.33	63.92	45.67	36.08	25.62	56.09	30.14	3.0	6.6	3.5	15.0	32.8	1.49	3.26	1.75	0.53	1.15	0.62
						1,300	54.33	62.66	45.67	37.34	26.03	56.98	30.02	4.1	9.0	4.7	13.3	29.1	1.64	3.59	1.89	0.57	1.24	0.66
	Aug. 20	1,281	1,035	15,445	665	1,413	53.83	62.84	46.17	37.16	25.09	54.35	29.30	4.6	10.0	5.4	14.3	31.0	1.46	3.17	1.71	0.62	1.35	0.73
						1,414	53.83	62.33	46.17	37.67	25.75	55.77	29.81			5.3	13.6	29.5	1.59	3.45	1.84	0.55	1.19	0.64
Warrendale, Ore., 130 miles	Aug. 5	1,261	873	8,100	524	1,241	53.31	61.72	46.69	38.28	26.49	56.74	30.67	4.2	9.1	4.9	13.6	29.2	1.59	3.41	1.84	0.73	1.57	0.85
						1,242	53.31	61.66	46.69	38.34	26.37	56.47	30.50			4.9	13.5	29.0	1.69	3.62	1.95	0.85	1.82	0.98
	Aug. 7	1,263	1,038	16,240	747	1,264	55.12	63.63	44.88	36.37	25.78	56.66	29.58			4.3	13.4	30.3	1.55	3.39	1.77	0.60	1.33	0.69
						1,265	55.12	63.76	44.88	36.24	25.35	56.49	29.33	3.8	8.4	4.3	13.6	30.2	1.65	3.67	1.90	0.56	1.24	0.64
Seufert's, Ore., 210 miles	July 12	1,056	851	7,625	465	1,101	54.26	65.18	45.74	34.82	25.43	55.60	30.55	1.8	4.0	2.1	16.7	36.6	1.23	2.69	1.48	0.50	1.09	0.60
						1,102	54.26	65.09	45.74	34.91	25.42	55.58	30.50				16.6	36.4	1.34	2.92	1.60	0.51	1.12	0.61
	July 13	1,060	955	11,065	715	1,111	50.04	61.00	49.96	39.00	25.84	51.73	31.51	3.4	6.8	4.1	18.0	36.0	2.10	4.20	2.56	0.61	1.24	0.75
						1,113	50.04	61.17	49.96	38.83	25.94	51.92	31.71				18.2	36.4	1.70	3.39	2.07	0.70	1.41	0.86
	July 31	1,180	863	7,835	570	1,199	51.91		48.09		25.93	53.92							1.78	3.70		0.58	1.20	

Ontario, Ore., 700 miles	Sept. 6	1,312	940	7,490	696	1,496	53.35	62.04	46.65	37.96	26.29	56.36	30.57	3.9	8.5	4.6	14.0	30.0	1.45	3.10	1.68	0.93	1.99	1.08
						1,498	53.35	62.67	46.65	37.33	26.13	56.02	30.70	3.4	7.4	4.0	14.9	31.9	1.27	2.73	1.50	0.92	1.97	1.07
	Sept. 7	1,315	1,076	10,410	916	1,523	54.59	61.41	45.41	38.59	28.57	62.92	32.14	3.5	7.8	4.0	11.1	24.5	1.52	3.34	1.71	0.64	1.41	0.72
						1,524	54.59	60.84	45.41	39.16	28.50	62.72	31.77	4.4	9.8	4.9	10.3	22.6	1.52	3.35	1.70	0.66	1.45	0.73
	Sept. 9	1,321	1,076	14,900	1,899	1,553	54.86	64.66	45.14	35.34	26.58	58.89	31.33	1.7	3.8	2.0	15.1	33.6	1.12	2.50	1.33	0.53	1.17	0.62
						1,555	54.86	64.39	45.14	35.61	26.52	58.75	31.13				14.8	32.5	1.40	3.10	1.67	0.68	1.50	0.81
	Sept. 11	1,326	1,034	12,840	1,282	1,579	54.64	63.93	45.36	36.07	27.01	59.54	31.60	1.7	3.7	1.9	14.5	32.0	1.42	3.13	1.66	0.71	1.56	0.83
						1,580	54.64	63.57	45.36	36.43	27.02	59.58	31.40	2.5	5.5	2.9	14.0	31.0	1.41	3.11	1.64	0.64	1.42	0.75
Cazadero, Ore., 130 miles	Aug. 25	1,294	957	9,060	1,775	1,429	57.24	65.23	42.76	34.77	25.89	60.55	30.00	2.3	3.1	2.2	12.2	28.6	1.57	3.67	1.75	0.76	1.77	0.86
	Aug. 26	1,296	1,054	12,910	1,860	1,452	57.68	65.60	42.32	34.40	26.66	63.01	30.33	1.5	3.5	1.7	12.1	28.5	1.41	3.34	1.60	0.66	1.56	0.75
					(ova)*	1,453	57.68	65.04	42.32	34.96	26.45	62.50	29.83	2.4	5.6	2.6	11.3	26.7	1.44	3.42	1.63	0.71	1.67	0.80
	Aug. 26	1,297	966	10,515	2,243	1,460	59.96	67.00	40.04	33.00	24.85	62.07	27.77	2.6	6.6	2.9	10.5	26.2	1.13	2.83	1.27	0.90	2.25	1.01
						1,462	59.96	66.01	40.04	33.39	24.87	62.11	27.63				10.0	24.0	1.77	4.43	1.79	0.77	1.92	0.78
	Aug. 27	1,299	887	8,145	1,679	1,473	58.19	64.02	41.91	35.98	27.06	64.73	29.78	3.6	8.6	3.9	9.1	21.7	1.37	3.27	1.51	0.65	1.55	0.77
					(ova)†	1,474	58.19	64.08	41.91	35.92	26.95	64.46	29.68				9.2	21.9	1.46	3.49	1.61	0.59	1.41	0.65

* The total weight was 2560 grams, the ripe ova free in the body cavity 1860 grams, and the ovary with adherent ova 736 grams.

† Free ova = 1679 grams. Ovary = 71 grams. Egg fluid = 179 grams. Total = 1929 grams.

The collections reported in this table represented the two Columbia river runs, the Ilwaco specimens are fall run fish and have more mature ovaries than those entering in April and May and collected at the Cazadero egg-taking station in September. The spring weights of ovary are 200 to 250 grams only.

The developing ovary contains a small and comparatively constant amount of glycogen or glucose. The amount ranges from 60 to 180 mgm. per cent, the latter figure was obtained from an actively feeding king salmon at Monterey Bay, California, hence probably has a relation to the intake of foods. The average content of carbohydrate was 90 mgm. per cent, which coincided exactly with the quantity obtained from the two samples of mature ova. This is less than one-third the carbohydrates reported by Kojo, 1911, for the yolk of the hen's egg, and only one-tenth that stored in certain invertebrate eggs, 1895.

The organic extractives are indicative of the mechanism of transfer of protein materials from the muscles to the growing ovaries. The extractives were relatively constant in the ovarian tissue throughout the fasting migration. The averages are 1.8, 1.86, 1.93, 1.61 and 1.59 per cent. One may assume a saturation in the ovarian waters, as proven for the salmon muscle by C. H. Greene, 1919. The average of 1.59 per cent at the spawning station on the Cazadero Creek, Oregon, was a lower quantity than found in muscle. The partition of the extractives for fish ovarian tissue has not yet been determined.

The total ash of the ovary was lowest at the beginning and is slightly augmented until the end of the migration, 0.7 per cent increasing to 0.8 per cent, computed as fat-free tissue.

The percentage of water in the salmon ovary is always low but slightly greater at the end of the journey, 58.27 per cent in comparison with 54.08 per cent at the beginning. The increase in water parallels that observed in the blood and in the salmon muscles, but it is less significant in amount. In fact the increase in percentage of water is general for all the various salmon tissues as the fasting migration proceeds. The growing ovary is not exempt from the operation of the mechanisms in the salmon that maintain the water balances during the tremendous enzymatic, oxidative and lytic processes incident to the great output of energy and tissue transformation during a fast so prolonged and exhaustive. In the light of such considerations, termination of the stress by death after liberation and discharge of the ova at spawning does not seem so inexplicable.

HISTOLOGICAL CHANGES DURING THE SPAWNING MIGRATION. Changes in the microscopic structure of the tissues of fishes during the spawning migration have been thoroughly investigated on two species only. Gulland, 1898, and Mahlanobis, 1898, studied the Atlantic salmon comparing the structures of the tissues at the beginning and at the end of the spawning migration. Greene, 1912, made a study of the king

salmon muscle extending the comparison to include intermediate stages as well as the extremes. These writers were searching for a cellular basis for the pronounced biological and chemical changes which characterize these great species, changes which in the king salmon terminate in death. The investigations have included the major tissues of muscle, reproductive organs, liver, digestive tract, etc. The chief interest lies in the structure of the muscle tissues and in the involutionary changes in the tissues of the alimentary tract.

The muscles. The different muscles types were examined from king salmon collected at all stages of development and of fasting during the migration, and including dying salmon. Evidences of changes in normal or pathological structure, distribution of microchemical fats and fat storage were presented in detail and are briefly reviewed here.

There are two distinct histological types of striated muscle in the king salmon. Both are represented in the great lateral muscle. These are the dark muscle, which is typified by the fibers of the superficial division of the great lateral muscle and by certain fin and cheek muscles. The second is the pink muscle, the white or pale muscle of most fishes.

The pink muscle has fine compact fibrillae, is finely striated and has a relatively small amount of inter-fibrillar substance, otherwise it is in no way unusual. Its stainable fats are largely stored in the intermuscular connective tissues during the active feeding. No fat has been observed within the sarcolemma, but extraordinary quantities are uniformly loaded into the intermuscular spaces. At the beginning of the fast minute chains of liposomes appear for the first time within the muscle fibers as deposits in the inter-fibrillar spaces. They are oriented with reference to the sarcomeres and discs of Henle, as described by Bullard, 1913, for the fatty infiltrations of pigeon muscles. The liposomes vary from 0.6 to 1.2 micra in diameter, rarely larger. They become more numerous and perceptibly larger during the intermediate stages of the migratory journey and are still present, but extremely small and fewer at the final spawning.

The dark muscle fibers, on the other hand, have coarser striations, larger sarcomeres and a much larger quantity of interfibrillar sarcoplasm. Neutral fats are also present in great quantity in the intermuscular spaces, but this tissue is unique in the amount of fat deposited within the fibers at all stages of development. Remnants of this fat are still present in spawning fish in the extreme stage of inanition.

The quantity of the intra-muscular fat of the dark muscle is so great at the height of the fat storage that it obscures the normal structure of

the fibers. Intra-muscular fat droplets may be as much as 10 or 20 micra in diameter. They spread the fibrillae apart for the length of several discs of Henle and many large droplets are laid close under the sarcolemma, a phenomenon observed and figured by Gulland, 1898, but not recognized as a characteristic of a special type of muscle until described for the king salmon. The larger deposits of 30 per cent and more of fats, the intra-muscular fat, does not destroy the normal tissue structure. But its presence does distort its structure and lower the physiological efficiency of the muscle.

The reduction of the fats of the muscle as the fasting migration proceeds materially alters its histological appearance. In the dark muscle the fat is rapidly withdrawn both from the inter- and intra-muscular deposits, and the liposomes become progressively smaller and disappear. The distribution and size of the liposomes of the dark muscle become more and more comparable to the intra-muscular liposomes of the pink muscle earlier in the migration.

Degenerating muscle. A very illuminating observation was made on the cheek muscles of a salmon collected immediately after spawning and at the approach of death. Fibers were found to be in the process of disintegration. The degeneration was shown by two features. The most extremely disorganized fibers had lost all structural characteristics and had reached the stage of Brownian pigmentation, the particles being scattered promiscuously through the fibers. In adjacent fibers, striation had entirely disappeared and the whole myoplasm presented the appearance of a structureless but swollen colloid. It contained no fat, had lost definite staining properties, and here and there isolated colloid-like droplets were present. This degree of degeneration was never observed in the great lateral muscle, yet it is suggested that it ultimately occurs more or less generally in dying salmon.

The alimentary tract. Rutter, 1902, published figures from salmon on the Sacramento River showing very pronounced gross retrogressive or degenerative changes in the size of the alimentary tract as the spawning migration progressed. This we have fully confirmed. The outstanding points are that at the very beginning of the fast the entire organ contracts into a firm mass with its lumen all but obliterated, in sharp contrast with the usual dilated stomach and intestine during the feeding stage. The stomach, for example, which is 18 by 7 cm. long in its two loops has at the time of spawning decreased to 5 by 2 cm. The resorptive change affects the size and structure of all the tissues. The columnar epithelial cells at first sharply decreases in length and other

dimensions and becomes small and cube-like before death. The mucous cells are at first more numerous, then disappear. Areas of the mucosa completely disintegrate with desquamation. The cell groups are often found as cell islands in the alimentary cavity. The cells of the gastric glands retain zymogen granules for a time but the granules are progressively resorbed and the glands atrophy and disappear.

The muscle fibers decrease in size and the cell nuclei become pycnotic as the resorption progresses. The formed cells, especially the granule cells become much more prominent by contrast. The changes parallel those shown by Gulland for the Atlantic salmon, but are more extreme in the alimentary tract of the king salmon.

In both the small intestine and the pyloric coeca these disintegrative changes are similar in character, more extreme in degree and often completely obliterate the cavity. The coeca of spawning salmon are mere solid threads in contrast with the normal.

The Atlantic salmon is known to regenerate or recover and feed again but the king salmon does not have this power.

The general atrophy extends to the diffuse pancreas which forms a net over and among the pyloric coeca. The islands of Langerhans are not involved to the same degree as the acinous tissue and seem correspondingly more prominent, though apparently they, too, decrease in absolute size.

The liver is affected by the retrogressive changes. Its volume decreases from 30 to 50 per cent and there are corresponding cellular atrophies. The spleen of all the visceral organs maintains its normal size and vascularity.

There are profound changes in the skeletal structures of the head; of the jaws and teeth, and of the skin as the fasting continues. The skin increases in thickness, its fat disappears and there is an augmentation of the subdermal connective tissues, clearly an adaptation to the fresh water environment. These changes rest on a morphological basis that has not yet been fully determined.

PHYSIOLOGICAL REACTIONS OF THE KING SALMON. Few tests of the usual type in dynamic physiology are possible with fishes. Such experiments have been limited almost exclusively to the heart rates, blood pressures, nerve reactions of the vascular system, and similar measurements of respiratory movements. It will be remembered that the discovery of the inhibitory influence of the vagus nerve on the heart, by the Weber brothers, 1846, was first demonstrated on fishes. The literature of the middle of the last century contains many interesting con-

tributions to the physiology of the heart and its nerve control in this group. The experiments of Greene, 1904, on the migrating king salmon are the only detailed experiments recording measurements of the circulatory system in the larger bony fishes.

Greene compared the blood pressures in marine and in spawning salmon. It is obviously very difficult to keep the large salmon alive and quiet while out of water. The conditions were met by binding the fish to a special canvas covered holder to control the violent muscular movements, and by supplying air by the method of running water through the mouth and over the gills.

Fig. 6. A blood pressure curve from the ventral aorta of the king salmon at the spawning beds on the McCloud River, California. The maximal pressure was 75 mm. of mercury. Time in seconds. From Greene.

In the circulatory system of fishes the gills are interposed between the great ventral aorta and the dorsal aorta with its numerous systemic branches.

Records were obtained of the blood pressures from the ventral and the dorsal aortae by the usual method of mercury manometer and arterial cannula. The pressures from the ventral aorta varied from 40 to 120 mm., with average of 74.6 mm. The blood pressure presented in figure 6 exactly coincided with the average. The pressure in the dorsal aorta varied between 44 and 55 mm. of mercury, an average of 53 mm. The average difference between the pressures in the two vessels was 21.6 mm.

Pressures taken from a number of females after artificial spawning varied between 57 and 120 mm., showing that the circulatory pressure of these spent fishes was still efficient and in no way depleted in energy.

The heart rate in the king salmon is comparatively slow, averaging only 59 per minute in a long series. The pulse pressure, on the other hand, is high, often amounting to 16 to 18 mm. of mercury.

The normal respiratory movements of the salmon continue for long periods under artificial aeration. Such rates vary from 60 to 120 per minute. The respiratory rate often coincides with the heart rate but it has no further significance.

The salmon heart is very sensitive to inhibitory nerve control. The

slightest stimulation of the skin results in a reflex which produces a more or less pronounced inhibition of the heart, also of the respiratory movements.

The data submitted on the physiological reactions of the circulatory and respiratory mechanisms are sufficient to indicate that these great systems are maintained in a high degree of efficiency, at least for a time after the spawning act. The heart itself is notably vigorous with a latent reserve of energy sufficient to produce a blood pressure of twice the normal amount. These data strongly indicate that the extreme exhaustion following the spawning act produces death through some other more vulnerable channel than the heart and blood vessels.

GENERAL DISCUSSION

The development of the ovaries in the migrating salmon is truly remarkable, especially so in view of the fact that it takes place during a period of complete starvation. Calculated on the spawning fish number 1297 of table 7, the 2243 grams of ovary contain 230 grams of fat and 558 grams of protein chiefly formed during the journey. This material which represents foodstuff as stored yolk for the young can come only from the tissues of the parent fish. Small amounts of material may be liberated in consequence of the involutionary changes in the alimentary canal, skeleton, etc., but the great bulk of the material came from the stores in the muscles. As with the pelican of fable, the young are fed from the tissues of the parent.

Not only are the muscles the chief store from which the material for the gonads is drawn but they also must provide from their own substance for prolonged activity. The spawning migration is a long and arduous journey against the force of the current of swift streams. One has only to watch these fish making their way up stream or leaping in the rapids to appreciate the splendid efficiency of the motor mechanism. This muscular activity is all the more remarkable when the impoverished state of the muscle itself is considered. Smallwood has reported muscular degeneration in the bowfin, *Amia Calva*, as a result of prolonged inanition. No such change takes place in the salmon until death is imminent.

In table 3 it is shown that a fish of 86 cm. length lost 589 grams of protein and 1177 grams of fat from the muscles during the migration. Reducing the weights of number 1297 above to the 86 cm. standard its stored fats and proteins are 161 and 390 respectively. The difference gives 199 grams of proteins and 1016 grams of fat to be accounted for.

This represents tissue used to supply energy for the migration. This change, while striking, in no way interferes with the functional efficiency of the muscle. Histological examination further shows that the structure of the active tissue is undisturbed. The fact that 30 per cent of the protein content of the muscle could be lost during the migration, table 5, led Greene, 1919, to the view that this is evidence for the presence in the muscle of stored or deposit protein. In the salmon this represents protein over and above the quantity necessary to maintain the integrity and activity of the muscular protoplasm. It is this protein which is consumed during the migratory journey. "Since the muscle remains physiologically active and vigorous the conclusion is drawn that the 30 per cent of muscle proteins lost from the normal, as shown by the composition of the spawning muscle, is stored protein which the salmon can use in the emergency of the migration without jeopardizing the protoplasmic integrity of the muscle."

Moulton, 1920, found that the muscles of beef cattle likewise contained stored or deposit protein. This conclusion was based on the observation that the muscles of full fed cattle contained 5 to 6 per cent more protein per unit of protoplasm than did the muscles of underfed animals. Recently Boothby, Sandiford, Sandiford and Slosse have presented evidence for the occurrence of deposit proteins in man. Boothby's data are derived by an entirely distinct and unique method. These two confirmations of the original hypothesis greatly emphasize the significance of the proteins as a reserve source of energy during the fasting period of the salmon spawning migration.

The present discussion of the energy exchange in the muscles has been confined to the changes in the protein and fats. This is necessarily so since carbohydrates apparently have little or no place in the internal economy of the king salmon. The usual tissues in which glycogen storage occurs in other vertebrates contain little or none of this food material. The total glycogen and carbohydrate content of the great lateral muscle of feeding salmon in Monterey Bay, California, is equivalent to but 14 to 15 mgm. of glucose per per 100 grams of muscle. The muscles of fish from Bolinus Bay, which probably were fasting, contained but traces of carbohydrates, and even these traces disappear early in the migration.

The maximum glycogen and glucose content of the liver was 700 mgm. per cent with an average of 380 mgm. In spawning fish from the McCloud River the carbohydrates in the liver were reduced on the average to 52 mgm. per cent.

The eggs of the developing ovary contain a small amount of stored glycogen. The quantity averages 90 mgm., but varies from 60 to 180 mgm. per cent, the higher values being obtained in actively feeding salmon. This quantity is not more than one-third the amount of carbohydrates found in the yolk of the hen's egg by Henze and only one-tenth the amount demonstrated in certain invertebrate eggs by Tichemeroff, 1895.

Preformed carbohydrate in the usual quantities is obviously not essential for the physiological activity of the salmon. Glucose may be derived from the metabolism of proteins, but even assuming the maximum formation of glucose from protein in the salmon the amount would still be insufficient for the complete metabolism of the fats according to the usual antiketogenic ratios advocated for higher vertebrates and man.

I have emphasized the point that the energy for the whole of the strenuous muscular activity involved in the spawning migration is derived from the bodily stores of fat and protein. In the absence of evidence to the contrary we may conclude that the salmon is able to metabolize these foodstuffs directly. This is an important point, for the present day concepts of energy exchange in muscular work, both in the frog and man, are formulated around the Hill-Meyerhof theory. The latter assumes that the energy for muscular contraction can be directly supplied only through the intermediary of the carbohydrates. The salmon muscle is deserving of further study from this viewpoint. In the apparent ability to utilize fats one must recognize either the presence of several at present unrecognized transformations, or the existence of a unique and characteristic physiological mechanism.

While it is impossible to discuss all these questions in full detail this presentation of the physiological changes during the spawning migration will emphasize the interest and importance of such studies in comparative physiology.

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THE PHYSIOLOGY OF THE SEROUS MEMBRANES

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In attempting to consider the general subject of the physiology of the serous membranes, it is important to note that it has become customary to consider the pleural, pericardial and peritoneal cavities as wholly similar in their functions and reactions. This generalization is supported by most of the evidence which has been obtained by the numerous investigations on the subject and will therefore be accepted as the basis of this review. It will be presented largely from the standpoint of the reactions of the peritoneum, but wherever there has been some evidence of differences in the other serous cavities this will be discussed. This method of approach has been selected because the peritoneum has been studied much more extensively than either the pleura or the pericardium. This preponderance of work on the peritoneum has been due to its greater accessibility and to the fact that it is involved so pertinently in many important surgical procedures. It is always a question as to how far the joint cavities, the bursae and the tendon sheaths should be considered as true serous cavities, but in as much as the work regarding their physiology is quite meager and indefinite they will not be considered here. The cerebrospinal spaces are likewise often considered as closely related to the true serous cavities, but the physiology of this system has been recently presented in this journal (Weed, 141) and will therefore be omitted.

The "physiology" of the serous membranes may be considered under two general headings: first, the relationship of the serosal lining cells to other cells, both to the cells which constitute the subjacent connective tissue and to the free cells which occur normally within the cavities and in exudates; and second, their reactions as membranes which divide the blood stream from the fluid occurring either normally or adventitiously in the serous cavities, thus including their power of absorption, transmission or secretion. Before taking up these two aspects of the function of the serous membranes it will be advisable to consider somewhat in detail the structure of the cells which constitute the functioning layer.

Structure of the peritoneum. The "peritoneum" is generally defined as a connective tissue layer made up of cells and fibers and covered by a layer of flattened "endothelial" or "mesothelial" cells. From a functional point of view this layer of cells is the essential part of the membrane in question and their character, relationships and reactions constitute the essential basis of the physiology of the serous membranes. In many of the discussions of the peritoneum such as Robinson's (119), Kolossow's (83) and Hertzler's (71) there is very elaborate discussion of the structure of the submesothelial connective tissue layer. But the finer structure of this layer is of relatively little importance in comparison with that of the essential mesothelial cell. I shall consider briefly some of the important points in the structure and arrangement of the surface cells (mesothelial elements) but will refer the reader to the articles listed above for consideration of the submesothelial layer.

The mesothelial cells have been in general called *endothelium* (His (73), (74); Schweigger-Seidel and Dogiel (126); Palladino (110); von Recklinghausen (116), (117)), but there have been others who have considered these cells as being more closely allied to epithelium than to endothelium and have therefore felt that these cells should be included in the epithelia (Waldeyer (137); Neumann (107); Bichat (6)). On the basis of the work of Minot (102) and of Mall (95) it seems to me better to apply the term *mesothelium* to the cells lining the serous cavities, and reserve the term *endothelium* for the cells lining the blood-vessels and lymphatics.

The peritoneal cavity has been known since the earliest records of the dissections of animals and is described, in the Ebers papyrus which was written about 3,000 years B.C., as a definitely outlined cavity in which the viscera are somehow suspended. All discussions regarding the structure and function of the peritoneal membranes consisted of the vaguest type of theorizing until late in the 19th century when the effect of the discovery of cells began to be felt throughout anatomy. Subsequently two main concepts have emerged from the chaotic theories of the earlier times; the one, that the peritoneal cavity is an integral part of the lymphatic system, and the other, that it is lined by a characteristic type of flattened cell. Upon these two concepts, which we owe very largely to the work of Bichat (6), Henle (67), and especially von Recklinghausen (116), (117), has been based all of the modern study of the lining of the peritoneal cavity. It was with von Recklinghausen's work that the modern epoch of the study of the peritoneum

began; for it was he who laid down the first constructive idea of the structure and function of the lining cells. Von Recklinghausen introduced the study of cellular membranes by means of the silver nitrate reaction and demonstrated sharply for the first time that the peritoneal cavity, as well as the blood vessels and lymphatics, is lined by thin, flattened cells whose margins could be made out sharply and characteristically. These margins, he found, were irregular but not so indented as those of the endothelial cells of the lymphatics. However, from his work, he considers the peritoneal cavity to be lined by these cells except in small areas where openings into the lymphatics occurred; these openings he called *stigmata* and *stomata*.

Dekhuysen (37) studied the serous membranes after impregnation with silver nitrate and described blind stomata at the junctions of the cell-borders wherever there was a radial arrangement of the cells; leucocytes could be seen in the bottom of these stomata. Beck (5) using this same method described short canals lined with endothelial cells in the peritoneal membranes. These canals he considers as opening out to the peritoneal surface on the one side and communicating directly with the underlying lymphatics on the other. This concept of open stomata between the peritoneal cavity and the lymphatics was also supported by Schweigger-Seidel and Dogiel (126), Ludwig and Schweigger-Seidel (91) and Klein (82).

Among the earlier writers who disputed this concept of preformed stigmata and stomata and who regarded the peritoneal cavity as entirely closed by an intact layer of cells were Afonassiew (3), Foà (55), Tourneux (135) and Tourneux and Herrmann (136). The latter investigators conclude that the figures, which are seen in silver preparations of serous membranes and which are interpreted by many authors as stigmata and stomata are really the results of a combination of the silver salts with deposits of serous fluids adhering to the surface of the membranes. Oedmanson (108) although one of the first to describe the rings and dots on the intercellular lines appearing in silver preparations of the peritoneum believes these rings and dots to be deposits of silver albuminate. Hertzler (68) injected a solution of silver nitrate into the peritoneal cavity of dogs in order to ascertain the reaction on the peritoneum *in vivo*. By the use of 1:1000 to 1:500 solutions of silver nitrate he obtained preparations in which the intercellular lines were thinner and more regular than when the peritoneum had been prepared in the usual way; in these studies he found no evidence of stigmata or stomata. He came to the conclusion that the dark spots

and rings so frequently described were the result of the technique and probably represented deposits of silver chloride.

The most elaborate investigation on the structure and function of the mesothelial cells is that of Kolossow (83) who developed a very interesting method for their study. This method consisted of staining the cells with osmic acid and later "developing" this stain with pyrogallic acid and tannin. In this way he produced very beautiful histological preparations of the peritoneal, pleural and pericardial mesothelia, and from the study of these preparations came to the following conclusions with regard to their structure and arrangement. He illustrates and describes the mesothelial cell as a double plate, the inner portion of which is smaller in diameter than the outer. The inner portion contains the nucleus, and is connected with the adjacent cells by fine anastamosing processes. The spaces between these processes form little intercellular canals, but these do not communicate directly with the lymphatic system. The outer plates are not connected with each other being merely contiguous at the surface margins. He considers these outer plates as especially adapted to withstand the friction which constantly takes place in the movements of the viscera. He found no evidence in his experiments for the existence of an intercellular cement substance. Kolossow thus describes the entire mass of the lower portion of the mesothelial layer as a syncytial structure. Furthermore, he found, in mammals as well as in amphibia, typical cilia covering many of the mesothelial cells. Many observers including Robinson (119), Muscatello (104), Beck (5), Prenant (113), (114) and others have confirmed Kolossow's observations with regard to the presence of cilia on the mesothelial cells. Other observers, however, have failed to find such cilia on the cells of mammals. Hertzler (71) in his review of this subject suggests that the structures interpreted by some workers as cilia are really fine, fibrinous processes which are produced when peritoneal surfaces, which have been in contact, are pulled apart.

Muscatello (104) studied the structure of the peritoneal membranes by means of various techniques including that of Kolossow. He concludes that normally the mesothelial cells of the serous cavities form a continuous layer in which there are no openings of any kind. In attempting, however, to explain the appearance which previous observers had described as stigmata and stomata he postulated a retraction of the deeper portion of the mesothelial cells while the superficial plate-like portion of the cells remained approximated. He says:

An manchen Stellen können durch Retraction des Protoplasma von zwei oder drei benachbarten Zellen rundliche Räume entstehen, welche an der Oberfläche von der Lamella superficialis überdeckt sind. Zwischen den Endothelzellen finden sich unter gewöhnlichen Verhältnissen hie und da Leukocyten.

Von Brunn (9) also describes a net-like arrangement at the base of the mesothelial cells. MacCallum (92) (93) agrees with Kolossow and Muscatello that there are no pre-formed openings into the lymphatics from the serous cavities and that the mesothelial cells are connected with each other by processes, but states that his preparations suggest the possibility that the technique has in many instances caused a shrinkage of the cells; the resulting gaps between them being very large. Hertzler (71) denies the existence of intercellular processes finding that there is a cement substance between the cells from which globular masses are formed when treated with silver nitrate or osmic acid. It is these masses which he thinks many workers have mistaken for intercellular bridges. The recent work of Lewis (86) on the formation of mesothelium in tissue cultures as well as on the arrangement of the supposedly syncytial mesenchyme, indicates that in both cases the cells are only in approximation and not actually connected by syncytial processes.

The reaction of the peritoneal mesothelium to vital dyes has been studied by Goldmann, Kiyono, Evans, Foot and others. Goldmann (60) (61) was unable to find any staining whatever of the mesothelial cells. Kiyono (80) figures and describes them as storing considerable amounts of carmine scattered diffusely throughout the cell while Evans and Scott (45) and Foot (56) both note that they only stain with a few, fine droplets of the dyes used and they suggest that this is a valuable differential point between mesothelial cells and phagocytic mononuclears. Cunningham (26) found that the mesothelial cells of the peritoneum stained in a very specific manner in different areas and that these characteristic arrangements of the stain could always be found in the appropriate regions. In the splenic mesothelial cells, for example, the stain was always collected in a small mass near the nucleus, an area of the cell which has for other reasons been noted as of especial physiological importance.

The majority of those who have studied the finer characteristics of the mesothelial cells agree that they are closely bound to each other by some means. Cytoplasmic bridges are described which, if they are true connections between the cells, would indicate that the entire mesothelial membrane forms a complete and continuous syncytium.

Others consider that a special and peculiar cement substance binds the separate and distinct cellular entities together into a complete membrane. But on the other hand the recent work of Lewis indicates that the mesothelial cells are merely in apposition to each other. This work of Lewis' taken in conjunction with the observations, to be discussed later, that the mesothelial cells round up and separate as the result of irritation, suggests that the most rational position to take at present is, that the mesothelial cells are distinct cellular entities but which may, however, have some very close type of association.

RELATION BETWEEN MESOTHELIAL CELLS AND OTHER CELLS. Turning now to the question of the relationship of the mesothelial cells to other cells, we have two main points to consider. The first of these is whether the mesothelial cell is, under all circumstances, a differentiated functional unit, which does not change into any other specific type of cell; or whether, as the result of appropriate stimulation, it may undergo metaplasia into some other type of cell with wholly new functions and adaptations. The latter concept has been held very widely by students of the peritoneum and close genetic and functional relationships have been believed to exist between the mesothelial cells on the one hand and both fibroblasts and free, mononuclear, phagocytic cells on the other. Some observers have accepted one of these relationships, some the other, and some both.

Relation of mesothelial cells to fixed connective tissue cells. The concept of the close relationship between mesothelial and connective tissue cells has been based largely upon the morphology of the two types of cells, but recent work on the specificity of mesothelial cells has been directed more toward those modifications which take place following irritations and injuries than toward an elaborate investigation of the normal structure of these elements. Many workers have observed that after irritation of any sort the mesothelial cells undergo changes in shape, size and arrangement, tending always to become more numerous and generally rounded up. Marchand (96) (97) (98) (99), Roloff (120), Cornil (24) and von Büngner (11) studied the effect upon the mesothelial cells of the introduction of various irritating and destructive agents into the peritoneal cavity and then followed the regeneration of the surface layer of mesothelial cells as well as the formation of new connective tissue. While differing in minor details in their observations they all conclude that the mesothelial cells and fibroblasts are functionally interchangeable. This view is also held by Hertzler (71) who, after studying the regeneration of the mesothelial cells and

the healing of wounds in the peritoneum, concludes that the new-formed lining cells are identical with the subjacent connective tissue cells and that their difference in appearance is merely the result of location. He also found no evidence that the remaining serosal cells proliferated at the border of denuded areas, but believes such areas are recovered by migrating connective tissue cells. And Schott (125), while directing his attention principally to the rôle which he believes the fibroblasts and mesothelial cells play in the production of the free, phagocytic elements, nevertheless notes incidentally that he is unable to separate these two "morphologically similar" types of cells.

On the other hand a few observers, notably Büttner (12), Mönckeberg (103), von Brunn (10), Hinsberg (72), Kiyono (80), and Cunningham (31) have maintained the specificity of the fibroblast and have denied any close biological relationship between it and the mesothelial cell. Büttner studied the peritoneum after the production of an acute peritonitis by the injection of bacteria, and also after a mild inflammation had been produced by the introduction of silver salts into the peritoneal cavity. He found that the cells forming the surface layer of the peritoneum were always quite different from those within the tissues and that these surface cells did not resemble fibroblasts in any way. However, Büttner says that he cannot state positively whether mesothelial cells become transformed into fibroblasts during the formation of adhesions or not, but inclines to the view that they do not. Von Brunn observing the effect of the introduction of sterile, foreign bodies into the peritoneal, pleural and pericardial cavities, found that, in all cases studied by him, cilia were easily to be detected on the mesothelial lining cells but never on any of the connective tissue elements. He describes the proliferation of the lining cells and of fibroblasts during the inflammatory processes and says the two types of cells can be clearly differentiated; he found some difficulty however, in distinguishing fibroblasts from very young lining cells but says that if careful attention is paid to details of cellular structure the differentiation is never impossible. The lining cells are described by von Brunn as rounding up during inflammation and showing a net-like arrangement at the base of the cells. In his opinion the regeneration of the serosal membranes takes place by proliferation of the remaining lining cells and new lining cells are never formed from fibroblasts. Hinsberg studied the effect of the injection of lycopodium spores on the peritoneum and describes the rounding up and desquamation of the serosal cells. In regenerative processes he found that the new connective tissue was formed

by the rapid proliferation of the fibroblasts and that the mesothelial cells played no part in this process; later, however, after the connective tissue had become organized, the remaining mesothelial cells proliferated to form a continuous layer over the surface of the connective tissue. Hinsberg concludes that there is no genetic relationship between the mesothelial and connective tissue cells and no transition from the one to the other. Kiyono studied the fibroblast by means of vital staining with carmine. He describes its wandering out into the inflammatory areas but in his opinion it always maintains its peculiar biological and morphological characteristics.

Cunningham (31) found that when the mesothelial cells were irritated they underwent progressive changes from flat to cuboidal and finally to tall, columnar cells. At the same time that this change was taking place there was a tendency, in the mesothelial cells of the peritoneum in general, to show a dispersion of the ingested dye throughout the cytoplasm, while the cells covering the spleen usually presented a concentration of the dye in the basal portion of the cells. Nevertheless this scattering of the dye and the coincidental increase in amount which was also noted, did not bring the cells to a state where they could be confused with the heavily loaded clasmatocytes. During inflammations the mesothelial cells became rounded up, and both because of the lessened surface area covered by each cell and because of the desquamation of many injured cells, there was a great proliferation and formation of new elements to cover the denuded areas. The lining cells, it was found, always attempted to cover the surface layer and, in this way, to fulfill their primary function of preventing the formation of adhesions. Cunningham concludes that the new cells are always produced from preëxisting mesothelial cells and that as long as the surface layer of these cells remains complete, notwithstanding any change in shape or size which they may undergo, adhesions do not develop. He found that the development of adhesions in all cases depended upon such widespread destruction of surface cells that the surrounding normal cells were unable to cover the denuded areas before they became adherent to adjacent surfaces. In reporting a series of studies on changes in the omentum produced by mild irritations Cunningham (27) states that

> it has become entirely clear that the serosal lining cells and the fibroblasts react differently both as regards their morphological appearance and the distribution of their vital dye-content. The serosal lining cells increase in thickness and are much more compact than the normal, flat, plate-like structures. They differ

considerably in size and shape, being round, oval, or square and some of them have a few, fine-pointed processes which vary in size but not in character. The vital dye-content is more diffuse than in the normal cells but there is never any dye to be seen in any of the fine processes. On the other hand, the fibroblast during irritation becomes an elaborately branched structure, forming a marked contrast to the compact serosal cell. The processes of the fibroblasts vary enormously in size and shape; some are long and slender, others branched into two or more subdivisions, and many have bulb-like enlargements, especially where they branch. The vital dye is distributed throughout these processes, the granules often being collected in the bulb-like enlargements.

Cunningham's work therefore indicates that during the course of a mild irritation the changes which take place in these two types of cells indicate a progressive separation as far as their morphological characteristics are concerned. And it seems most likely that while we may be justified in considering the mesothelial cells and the fibroblasts more closely related to each other than either of these two groups are to the phagocytic mononuclears, yet they form two distinct groups whose morphological characteristics suggest different physiological activities.

Relationship of the mesothelial cells to the free cells of serous exudates. Turning now to the consideration of the relationship of the mesothelial cells to the free phagocytic mononuclears, which are always present in the peritoneal cavity and which are greatly increased during irritations, we find that the majority of the observers have looked upon mesothelial cells as an important, if not the only, parent-stem from which the exudate mononuclears are derived. Weidenreich (144), and Schott (125) have been the main exponents of the derivation of free phagocytic cells from the lining cells of the peritoneum though they have in addition, as noted above, considered mesothelial and fibroblastic elements as being jointly responsible for this production of phagocytic cells. Schott has presented evidence, all of which is based upon morphological appearances in fixed material, indicating that the lining cells and other sessile elements of the omentum enlarge, round up, and eventually become free in the peritoneal fluid. This he considers as demonstrating that these cells are the progenitors of the free, mononuclear, phagocytic cells of the exudate. Schott's conclusions have been supported by the similar observations of Heinz (66), Szecsi (132), Szecsi and Ewald (133), Downey (40) (41) and Marchand (97). Heinz's observations are particularly interesting in the light of recent work as he was the first person to describe the highly refractive droplets which have lately assumed so great an importance in separating the mesothelial cells from other types of cells in inflammatory con-

ditions. But Heinz did not recognize this fact as of great importance and failed to note any differences between the mesothelial elements which had become free and the true phagocytic mononuclear cells of the exudate.

The most ingenious experiment which has been carried out in attempting to analyse the origin of phagocytic mononuclear cells was that devised by Lippman and Plesch (87), (88). These investigators found that the administration of thorium-X brought about a depletion of the hematopoietic centers so that a relative aleucocytosis of the blood stream could be obtained. In some of their animals they were able to produce a leucopenia with as few as 600 cells per cubic millimeter. In such animals they produced peritoneal exudates by the exhibition of swine erysipelas into the pleural cavities and found that in these experiments the exudates consisted almost wholly of mononuclear cells in contrast to the large numbers of polymorphonuclear leucocytes obtained from the normal animal. They describe these mononuclear cells as showing every possible stage of transition from pleural mesothelium to cells which resemble small lymphocytes. They also found the pleural cells rounding up, becoming loosened from their connections with the subjacent tissues and giving rise to large, mononuclear, nongranular cells, which, because of their phagocytosis of red blood cells, they identified as macrophages. In order to eliminate the possibility of the derivation of exudative mononuclear cells from adventitial and perivascular cells they introduced aleuronat inter-muscularly. When this experiment was carried out in normal animals an exudate rich in all types of blood cells was obtained but in animals which had been rendered aleucocytic by the administration of thorium-X the injection of aleuronat produced no exudate whatever. When irritants were introduced into the peritoneal cavity of aleucocytized animals they found an exudate which differed from that produced in the pleura in that it contained typical polymorphonuclear leucocytes in addition to the mononuclear cells. They found no transition forms between the serosal cells and these granulocytic elements and conclude that there must be cells in the omentum analogous to the "hematoblasts" and "leucoblasts" of the bone-marrow.

On the other hand, the view that the free phagocytic cells of the peritoneal cavity are not derived from the serosal elements has been supported by Pappenheim, Evans and Cunningham. Of all the work done on peritoneal exudates that of Pappenheim (111), and of Pappenheim and Fukushi (112) is preëminently the best. Though Pap-

penheim has added unnecessary and even confusing details to the classification of cell types and cell genealogies, he has, however, made the most accurate observations on fixed material which have been made in hematology. Pappenheim studied the serosal exudates by various methods;—he produced exudates in otherwise normal animals and studied the cells with a great variety of hematological methods, he studied the effect of mild irritations on the serosal membranes in animals which had been previously stained with vital dyes, and then combined these methods with the Lippman-Plesch thorium-X treatment. Pappenheim became convinced that the lymphoid cells of the serosal exudates, in animals treated with thorium-X, were not derived from the cells of the circulating blood. He suggests that the stem-cells of the hematopoietic tissues, with which he includes hypothetical cells of a primitive type in the connective tissues, from which he believes the exudate cells are derived, are more resistant to the effects of thorium-X than the more highly differentiated forms of the bone-marrow and circulating blood. He considers that the serosal lining cells, lymph- and blood-vascular endothelium and polyblastic clasmatocytes all form possible sources from which the free exudate-cells may be derived. He found evidence in his experiments, however, which led him to conclude that the serosal cells do not participate in the formation of true phagocytic exudate cells. In the first place he does not believe that the cells lining the pericardial cavity and subarachnoidal spaces are true serosal lining cells comparable to those of the peritoneal and pleural cavities, because he found that, in a comparative study of inflammatory reactions in these four localities, exactly similar exudative cells were present in all. Secondly, in animals which had been vitally stained before the inflammatory reaction was produced, the exudate was always found to contain vitally stained cells, while the serosal cells showed no vital stain. And finally, he describes the vitally stained cells of the omentum as being very numerous and as making up the largest part of its cellular constituents, but these cells he considers as being clearly differentiated from the serosal cells since he found no transition forms between them. True serosal cells were always present in the exudate in his experiments but these in no case showed the same reaction as the usual phagocytic mononuclear cells. Pappenheim considers that the endothelium of blood vessels cannot be excluded as a possible source in the formation of histiocytes, but he found no positive evidence in his experiments on this point. Thus only the polyblastic clasmatocytes and adventitial cells of the omentum

and subserosal tissues remain as possible sources for the exudate-cells, and it is to these that Pappenheim refers their origin. This conclution is based upon certain morphological and physiological similarities in these two groups of cells. Both the free cells of the omentum and the large cells of the exudate have the same tinctorial reaction in fixed preparations, similar nuclear conformation, comparable type of ameboid movement, identical reaction to vital stains, and similar phagocytic capabilities.

Evans and Scott (45) in their elaborate study on the reactions of the connective tissue cells to vital stains have noted the fact that the peritoneal mesothelial cells stain only lightly with trypan blue and they suggest that this differentiates them sharply from the macrophages of the peritoneal exudates. Finally Cunningham (28) has studied the formation of peritoneal exudates using the supravital method of staining in conjunction with the use of carmine and trypan blue as vital dyes. We have already referred to the especial characteristics which the mesothelial cells manifest in their methods of storing such vital dyes as carmine and trypan blue. Utilizing these points of differentiation, together with the facts that, when mesothelial cells are irritated, they develop within their cytoplasm certain highly refractive droplets, and that they react in a particular manner to such supravital dyes as neutral red, Cunningham came to the conclusion that the desquamated serosal cells, under all circumstances, undergo degeneration and do not function as free, active, phagocytic mononuclears. The particular points of differentiation which he describes as characteristic of the mesothelial cells are, first: the small amounts of vital stain which they store, even in the most active state of proliferation; second, the large numbers of highly refractive droplets in their cytoplasm;[1] third, the presence of fine, red granules, scattered diffusely throughout the cell when stained supravitally with neutral red. In this study Cunningham made no attempt to differentiate between the various phagocytic cells which occurred in the peritoneal cavity, accepting the concept of Kiyono that both monocytes and clasmatocytes belonged to a single group of highly phagocytic cells. Later, Cunningham, Sabin and Doan (32) were able to differentiate two distinct types of phagocytic cells in the spleen of the rabbit. They found that these cells could be sharply divided into *monocytes* on the

[1] These droplets were found to stain a greyish brown in osmic acid, pink in Sudan III but did not react to Nile blue, so that it seems probable that they are, at least in part, made up of complex fatty substances.

one hand and into *clasmatocytes* on the other on the basis of their reactions to supravital dyes. With this concept in mind Sabin, Doan and Cunningham (122) restudied the problem of the cells in the peritoneal cavity and demonstrated that they could likewise be separated into two sharply distinct classes. They were wholly convinced, however, that the serosal cells under no circumstances gave rise to any of the free, phagocytic, mononuclear cells of the exudate.

THE ABSORPTION OF PARTICULATE MATERIAL. Von Recklinghausen's work initiated the modern period of the study of the absorption of particulate material from the peritoneal cavity. He found that when he stained the diaphragm with silver nitrate, there were areas which were not covered by the typical cells but seemed to be entirely denuded. Such areas appeared only as brown spots. These spots he considered to be actual openings between the lining cells that communicated with the subjacent lymphatic vessels and represented pathways by which substances could flow from the serous cavities directly into the lymphatic system. Von Recklinghausen (117) further reported that he had actually watched the substances flowing readily through these openings into the lymphatics in the diaphragms which had been removed and studied under the microscope. This theory of von Recklinghausen's, as we have already seen, was accepted by the majority of the early workers on the peritoneum and was only disputed by a very few until the careful and painstaking work of MacCallum (92), (93). MacCallum studied the absorption of particulate matter from the peritoneal cavity by a series of experiments which resulted in demonstrating conclusively and finally that the concept of stigmata and stomata, existing as normal and physiological openings between the peritoneal cavity and lymphatic system, was wholly unsound. Ludwig and Schweigger-Seidel (91) confirmed von Recklinghausen's experiments by finding substances, which had been injected into the peritoneal cavity in the anterior mediastinal lymph glands. Auspitz (4) also obtained confirmatory evidences by injecting ground rice into the peritoneal cavity. He found that after one hour the rice grains had passed through the diaphragm and could be obtained from the blood of the ear; still later he demonstrated them in the lung and the spleen. Beck (5) demonstrated that material injected into the peritoneal cavity could be found later in the thoracic duct. Dubar and Remy (43) claimed that other areas besides the diaphragm were important in absorption from the peritoneal cavity. Maffucci (94) and Muscatello (104) studied the effect of posture on the absorption of colored materials

from the peritoneal cavity and found that, when the animal was kept in such a position that the pelvic peritoneum was pendent, there was very little absorption through the diaphragm and though a small amount of the material could be found in the anterior mediastinal nodes it was relatively much less than when the animal was in an ordinary position. A few granules were, however, found in the abdominal organs. Maffucci concludes from this that other areas of the peritoneum are involved in the absorption of granules. Muscatello, however, came to the conclusion that, so long as the serosal membranes remain intact and in a fairly normal condition, the diaphragm is the only portion of the peritoneum involved to any extent in the absorption of particulate matter and that the mediastinal nodes serve to collect the lymph draining from the peritoneal cavity. He concludes that any granules found in the abdominal lymph-glands reach them by the lymphatic or blood-vascular route and not by direct absorption through the peritoneum.

MacCallum (93) attempted to find out the actual mechanism by which particulate matter passed from the peritoneal cavity to the lymphatic system. It had long been known that if a suspension of lamp-black or cinnabar were introduced into the peritoneal cavity, it would quickly appear in the anterior mediastinal nodes and later be distributed throughout the body. MacCallum restudied the question of the absorption of particulate matter and attempted to repeat von Recklinghausen's experiments. He found no evidence that material placed upon the eviscerated diaphragm flowed into submesothelial spaces through stigmata and stomata, and injections of the lacunae of the peritoneum with air and colored solutions proved, beyond doubt, that they were completely lined by a thin layer of cells. MacCallum was able to demonstrate large, phagocytic, mononuclear cells loaded with the exhibited substance making their way between the mesothelial cells into the lumina of the lymphatic lacunae which approach so closely to the surface of the diaphragm. He found also that many particles could be demonstrated within the cytoplasm of the mesothelial cells, but while he concluded that both of these mechanisms were active in the transference of particulate matter from the peritoneal cavity to the diaphragmatic lymphatics he thought that this transference was effected in much larger measure by a third mechanism—namely, respiratory movements. In order to more specifically determine the possibility of a purely physical transfer of particulate matter, as against that brought about by the agency of living cells, he performed an interesting series of experiments. Animals were sacrificed and kept in

the ice-box over night and the following day a suspension of India ink was introduced into the peritoneal cavity after which artificial respiration was carried out; after a given period of time the animals were opened and the diaphragms examined. When studied under the binocular microscope the diaphragms from these animals showed particles of particulate matter arranged in a pattern quite similar to that shown after the application of silver nitrate, i.e., the mesothelial cells were outlined by lines of particulate matter. In sections from these diaphragms he found interstices between the mesothelial cells, and these were everywhere filled with particles of injected material. MacCallum's conclusion was that the pumping action of the diaphragm caused a separation of the lining cells allowing the particles to pass easily between them and thus gain entrance into the subjacent layer. Thus MacCallum eliminated the concept of preformed physical openings advanced by von Recklinghausen, and substituted instead potential physical openings dependent upon the contractility or lack of expansive power on the part of the mesothelial cells.

MacCallum ignored the question of the absorption of particulate matter via routes other than that of the diaphragm. This is easily understood since it is wholly obvious that in all cases the vast majority of such material does leave the peritoneal cavity by this route. It has been suggested by Buxton (13), (14), and Buxton and Torrey (15), (16), (17), (18) that the omentum also represents a pathway for the removal of particulate matter. Their observations on this subject, however, are vague, for they speak of finding lamp-black in the afferent plexuses of the lymph-nodes of the omentum following its injection into the peritoneal cavity. Since there are no known lymph-glands in the omentum (Casparis (19)) it is difficult to know just what their observations represent. On the other hand, Shipley and Cunningham (127), (128) were able to demonstrate that particles of the finer sizes, at least, passed through the mesothelial layer of the omentum and the endothelium of its blood vessels; thus obtaining entrance to the circulating blood. These observations will be referred to again in considering the absorption of true solutions.

Cunningham (29) undertook to analyze the absorption of particulate matter via the diaphragm in an attempt to determine whether MacCallum's conclusion was correct with regard to the importance of the passage of materials between the mesothelial cells. He studied the passage of carbon granules through the diaphragm and was unable, in the normal animal, to find any evidence of the peripheral marking

of the mesothelial cells by the injected material. These experiments were carried out by the introduction of approximately 30 cc. of a mixture consisting of avian erythrocytes, large carmine granules, and very fine lamp-black granules in isotonic salt solution. This mixture was introduced into the peritoneal cavities of a number of cats of approximately the same size. These animals were killed at intervals varying from 1 to 30 minutes, and the diaphragms and the mediastinal lymph glands were examined histologically. It was found that after three minutes' exposure all three types of material had reached the mediastinal glands; all the particles seen were entirely free and had not been, in any case, taken up by cells. With increasing increments of time more and more particles were found inside of both free and fixed cells, but the greater part of the avian erythrocytes still remained free in the sinuses. Even at the end of thirty minutes very few phagocytic cells were seen in the lacunae of the diaphragm and it was evident that during the first thirty minutes very little of the particulate matter introduced into the peritoneal cavity had been removed by the phagocytic activities of free wandering cells. In his histological examinations of the diaphragms from these experiments Cunningham found that the mesothelial cells lining the peritoneum and the endothelial cells lining the lymphatics contained numerous particles of ink and carmine. He was unable to find mononuclear cells containing phagocytized material passing into the lacunae of the diaphragmatic lymphatics. Finally, he found that all along the surface of the diaphragm there were large numbers of granules and some red blood cells tightly adherent to the peritoneal surface of the lining cells. The granules which were within the cytoplasm of the mesothelial and endothelial cells usually occupied an irregular perinuclear zone. The most significant finding in these experiments was the occasional presence of a typical avian red blood cell obviously within the cytoplasm of the endothelium. From these experiments he concludes that at least a large proportion of the particulate matter passes through and not between the cells in the course of its absorption into the diaphragmatic lymphatics from the peritoneal cavity; and that, for the first thirty minutes after its introduction, this transfer of particles is not mediated by free phagocytic cells, though their intervention at a later time is not to be questioned.

In an attempt to analyse still further the factors involved in the passage of particulate matter through the mesothelial cells, Cunningham (30) studied this phenomenon in the fetus. It was hoped that

the conditions there would be sufficiently simple to permit a more careful analysis of the part played by the cell itself. The experiments were carried out on pregnant cats. A small amount of lamp-black was injected into the fetuses in utero without incision of the uterine wall. The fetuses were removed at appropriate intervals and killed as quickly as possible in order to forestall the initiation of any respiratory movements. In examining the material from these experiments it soon became evident that absorption by the diaphragmatic lymphatics had not taken place to any marked extent in the earlier stages. In the older fetuses the amount and rapidity of absorption increased consistently until in those approximately at term a very appreciable amount of absorption was found to have taken place though even in these a greater amount of time was required for the absorption of a given amount of material than in newborn kittens. While the exact time has not been established at which the fetus begins to make spasmodic respiratory movements, Cunningham reports that none under 75 mm. in length were ever seen to do so, even when watched, while still attached to the umbilical cord, for a considerable period of time. This group of experiments demonstrates that the respiratory movements are of importance in this connection, and suggests that one important factor in the absorption of particulate matter through the diaphragmatic mesothelial cells is by means of the pressure of the diaphragm on the intra-abdominal viscera.

In general, then, we may summarize the work which has been done on the mechanism involved in the absorption of particulate matter from the peritoneal cavity in the following way. The earlier work all tended to establish the concept of the presence of actual preformed physical openings between the peritoneal cavity and the diaphragmatic lymphatics. This idea was gradually eliminated and in its place the concept of potential physical openings between the cells was offered. In turn this hypothesis is being replaced by one which assumes that most, if not all, of the particulate material that is absorbed from the peritoneal cavity passes directly through the living cytoplasm of the mesothelial cells.

Absorption of solutions. We have seen how, as a result of a large series of investigations, the conclusion has become definitely established that particulate material is absorbed from the peritoneal cavity into the lymphatic vessels, and it has also been shown that the transference of this material has taken place, at least in large measure, directly through the living cytoplasm of the mesothelial and endothelial

cells. It is even more important, however, for us to consider the reactions which take place when true solutions and colloidal suspensions, whose particles are of small dimension, obtain entrance into the peritoneal cavity. This is true because there is always present in the peritoneal cavity a small amount of fluid, because this fluid is probably being absorbed and replaced with considerable rapidity, and because the question of the introduction of fluids into the peritoneal cavity is one of great importance from a physiological and therapeutic standpoint.

In the consideration of the relationship which the mesothelial cell bears to the removal of fluid from the peritoneal cavity, there are three main problems to be considered. In the first place it is important to know, as we have already seen with regard to the absorption of particulate matter from the peritoneal cavity, whether the fluids absorbed pass through or between the cells. Secondly, the question as to whether this absorption takes place via lymphatics, blood vessels or both, must be considered. Thirdly, it is essential to analyse what the mechanism is by which this absorption takes place; is it mediated wholly by the physical factors of osmosis and diffusion, or are there other factors, inherently resident in the lining cells themselves, which play a part in controlling this absorption?

The passage of solutions through the mesothelial cells. It is interesting to note that, in the consideration of the absorption of fluids and solutions from the peritoneal cavity, the principal subjects of discussion have been first of all, the routes by which the fluids escape from the peritoneal cavity and the factors involved in producing the absorption rather than the specific relationship of the mesothelial cell to these processes. This is in sharp contrast to the study of particulate matter where the rôle of the mesothelium has been the mooted question. This discrepancy is not surprising, however, when one considers how little is known about microchemical reactions, and that the study of absorption has been largely dealt with by means of more general methods. It was rather generally accepted, following the work of MacCallum, that all substances passed between the mesothelial cells in being absorbed from the peritoneal cavity, until Shipley and Cunningham (128) demonstrated that solutions of iron ammonium citrate and potassium ferrocyanide passed directly through the cytoplasm of both mesothelial and endothelial cells while being absorbed into the blood-vessels of the great omentum.

Routes of drainage of the peritoneal cavity. In general the earlier

workers on absorption of fluids from the peritoneal cavity accepted the view that the peritoneal cavity is a part of the lymphatic system and with this general hypothesis was included the idea that absorption took place necessarily into the lymphatic system. This concept was not seriously questioned until after the important work of Heidenhain (65) on the secretion of lymph indicated that the absorption of fluid from the peritoneal cavity takes place by means of the blood-vessels, the lymphatics having only a minor share. This work was confirmed by many investigators, particularly Starling and Tubby, Leathes and Starling, Orlow, Cohnstein and Hamburger.

Starling and Tubby (131) introduced dyes into the abdominal cavity and recorded the time necessary for these substances to appear in the thoracic duct and in the urine. They found that the dye appeared much earlier in the urine than in the lymph, and they conclude from these findings that absorption takes place through the blood vessels. They also suggest that the appearance of a small amount of dye in the lymph can be accounted for equally as well if the dye is considered to be secondarily transferred to the lymphatics from the blood stream. They arrived at this conclusion on account of the observation that the amount of coloration obtained in the lymph in these experiments can be produced just as readily by direct introduction of the dye into the blood stream. Heidenhain (65) introduced a specific quantity of fluid into the peritoneal cavity, allowed it to remain for several hours and removed the residue remaining at that time. He found that in every case a considerable amount of fluid had been absorbed during the experiment but that the lymph, which was obtained by means of a cannula (inserted into the thoracic duct before the beginning of the experiment), did not increase appreciably in amount. These observations were confirmed by Cohnstein (23), Orlow (109) and others. Hamburger (62), (63) studied the effect of the ligation of the thoracic duct upon the absorption of fluids of different osmotic pressures and came to the conclusion that these substances are absorbed via the blood vessels and that the obstruction of the thoracic duct has little or no effect upon the rate of absorption from the peritoneal cavity. These experiments all tended in the same general direction but none of them were sufficiently definite to decide conclusively whether the absorption from the peritoneal cavity takes place via the lymphatics or the blood vessels.

Adler and Meltzer (1), (2) point out the meager number of experiments which are recorded by the observers mentioned above and ques-

tion the accuracy of their results. The experiments of Adler and Meltzer were carried out with strychnine and with potassium ferrocyanide, the identification of which is relatively easy. Their experiments were performed on rabbits and since, in this animal, they found that operations directly upon the thoracic duct were extremely difficult, they tied off the left innominate vein. In the experiments with strychnine they found that the animals, in which this vein had been ligated with consequent obstruction of the thoracic duct, developed the typical symptoms of poisoning much more slowly than did those in which the thoracic duct was not obstructed. They further found that when potassium ferrocyanide was introduced into the peritoneal cavity its appearance could be detected much more quickly in the urine of animals whose thoracic ducts were functioning than in those in which this structure had been obstructed. Their series of experiments were very large and included many in which the question of cerebral anemia was controlled by the ligation of the jugulars, as well as experiments on the absorption of fluids from the cavities of dead animals. Thus these observers returned to the earlier concept of absorption and conclude that it takes place primarily via the lymphatic channels and that the blood vessels have but little or no importance in this mechanism. This view was also supported by Nakashima (105) who studied the absorption of fats from the peritoneal cavity and concludes that they are absorbed directly into lymphatics and not into the blood stream. Meltzer (100) studied the absorption of potassium ferrocyanide in dogs; he collected the lymph by a cannula in the thoracic duct and the urine by means of a catheter introduced into the bladder through an external opening in the urethra. The ferrocyanide solution was introduced into the peritoneal cavity by means of a blunt nosed syringe through a small incision. Meltzer found that in all of his experiments the characteristic Prussian blue reaction appeared in the lymph distinctly earlier than in the urine. He also introduced colored solutions into the peritoneal cavity and found that the color invariably appeared in the lymph earlier than in the urine. Mendel (101) repeated the experiments of Meltzer using indigo carmine solution as an indicator and found that this color appeared in every case earlier in the urine than in the lymph. Meltzer had explained that the results in Starling and Tubby's experiments were probably due to the fact that some of the colored fluids had obtained adventitious entry into the bladder via the incision in the abdominal cavity, the connective tissue spaces in the abdominal wall, and the wound where the

cannula was introduced into the bladder. On the other hand Mendel explained the results of Meltzer's experiments by suggesting that Meltzer's method of collection of urine permitted a considerable stagnation and thus accounted for the later time at which he found dyes present in the urine.

The question as to whether or not substances pass directly into the lymphatics has been definitely settled as far as particulate matter is concerned. There likewise seems to be no reason to doubt that solutions may also be absorbed to some extent in a similar manner. But while the weight of the evidence obtained from the experiments which have been described above, indicates that the blood vessels also represent a pathway by which fluids escape from the peritoneal cavity, this was not conclusively demonstrated until the observations of Dandy and Rowntree and of Shipley and Cunningham. Dandy and Rowntree (35), (36) studied the absorption of phenolsulphonephthalein from the peritoneal and pleural cavities and found that it appeared much more rapidly in the blood and urine than in the lymph of the thoracic duct. They summarize the exact details as follows (35):

> The results following the intraperitoneal injections of phenolsulphonephthalein may be summed up as follows: 1. The appearance time in the *blood* is 2–4 minutes.
> 2. The appearance time in the *urine* is 4–6 minutes.
> 3. The appearance time in the *lymph* (thoracic duct) is 20–60 minutes.
> 4. The quantitative output in the *urine* for 1 hour is 40–60 per cent.
> 5. The quantitative output in the *lymph* for 1 hour is less than 0.1 per cent.
>
> It can therefore be seen that the lymph plays practically no part in the absorption of these fluids.

Shipley and Cunningham (127) demonstrated that when the omentum was withdrawn from the peritoneal cavity through a mid-line incision and immersed in solutions of iron ammonium citrate and potassium ferrocyanide these substances passed directly into the blood stream and were excreted by the kidneys. In order to obviate the possibility that some of this absorption had taken place via lymphatics in the omentum, the authors ligated the thoracic duct without affecting the rapidity or amount of the substances entering the circulation as indicated by the amount of the Prussian blue in the urine. Furthermore they demonstrated that these substances were passing into the blood vessels by finding the granules of precipitated Prussian blue in the lumina of the capillaries and in the endothelial cells themselves.

Thus it now seems wholly settled that solutions which are absorbed from the peritoneal cavity pass in large part directly into the blood

stream. It is, however, quite probable that a small amount of this material is also absorbed through the lymphatic vessels. The chances are, however, that this quantity is very small, since it is probable that the rate of absorption is dependent to a great degree upon the rate of flow through the blood vessels. Since the rate of blood flow is so greatly in excess of the rate of flow in the lymphatics only a small amount can possibly escape by way of the latter vessels.

The mechanism involved in absorption. The subject of absorption has been of very great interest because, in the passage of substances through living membranes, an opportunity has been offered for an application of those physical principles which have been of so much interest during the last fifty years. Of these principles the laws of diffusion and osmosis have been the two which have been depended upon in large measure by those investigators who have attempted to reduce the principles of absorption to known physical forces. The modern era in the study of absorption was introduced by Heidenhain's classical observations on absorption from the intestine. With these observations as a model many observers have approached the subject of the peritoneal cavity in a similar manner. Heidenhain himself began experiments on the peritoneal cavity which were elaborated and completed by Orlow. Orlow (109) studied in great detail the absorption of various solutions from the peritoneal cavity, using hypertonic, hypotonic, and isotonic solutions as well as blood serum. He found that hypotonic solutions were absorbed in approximately inverse ratios to their hypotonicity, i.e., the less the concentration of the solution the more rapidly it was absorbed. Furthermore he found that hypertonic solutions became more dilute by passage of fluid from the blood stream into the peritoneal cavity until an isotonic condition obtained. After this isotonic balance had been established absorption took place more slowly. From these experiments Orlow concludes that fluids and salts obey the laws of osmosis and diffusion until an osmotic balance is obtained after which absorption takes place through the agency of the physiological activity of the lining cells themselves. Somewhat similar results have been obtained by Roth (121). He studied the absorption of fluids from the peritoneal cavity and noticed particularly the effect of sugar and urea when introduced in different concentrations into the peritoneal cavity. He also studied the absorption of solutions of albumen which were obtained by long-continued dialysis of horse serum. Roth emphasizes the effect of the specific substances on the passage of fluids through the cellular membrane and

maintains that the balance obtained is always measured by the relative permeability of the vascular wall to these specific substances. For example he found that a solution of sugar brought about a much greater outflow of water into the peritoneal cavity than a solution of urea having the same effect upon the lowering of the freezing point. Thus he emphasizes the relativity of the permeability of the blood-vessels (and in this he includes the mesothelial layer, i.e., he does not discuss the two cellular layers separately). Roth does not specifically commit himself on the mechanism involved in the removal of a completely balanced isotonic solution, but indicates that the rapid changes of the contents of the blood vessels makes it possible for the diffusion coefficient to become so altered that an isotonic solution may be absorbed.

Starling and Tubby (131) investigated absorption from the pleural cavity by introducing colored solutions, lymphagogues and solutions of sodium chloride of varying tonicity. They found that the dyes used appeared much more quickly in the urine than in the lymph and that they never appeared in the latter in appreciable quantities. They did however note, in some of their experiments, that the anterior mediastinal lymph glands were definitely stained. They also found that when substances of different tonicity than the blood were introduced, an osmotic interchange took place until the fluid in the pleural cavity became isotonic with the blood. Subsequently absorption still took place, but at a relatively slower rate. They state:

> it is evident from these experiments that although a great excess of osmotic pressure in the pleural cavity can determine a flow of fluid from the blood into the cavity, yet if the difference between the osmotic pressures in the pleural fluid and blood is not excessive, fluid will be taken up by the blood. The cells between the blood and the pleural fluid seem to exert a pull on the latter; in fact there is an *active* absorption going on. The cells must perform a considerable amount of work in this absorption, but the work they can do has a limit. If this limit be passed then the passage of fluid follows the osmotic difference. So that we must look upon the living cells as being actively concerned in the absorption of a fluid such as 1 per cent saline.

Leathes and Starling (85) tried to analyse the mechanism involved in the absorption of the isotonic solution which Starling and Tubby had been unable to determine, modeling their experiments after the classical experiments of Heidenhain on absorption from the intestinal tract. They introduced isotonic solutions into the pleural cavity and then determined the Δ of the fluid and blood serum at various intervals.

These investigators also studied the amounts of the solute remaining in the recovered portions of the injected fluid. From these experiments they conclude that the substances in the injected fluid gradually pass into the blood but that the fluid remains approximately isotonic as a result of the passage of other substances into the fluid from the blood. Furthermore, they found that if they added sodium fluoride to the substance introduced, there was no change in the rate of absorption. They had reasoned that, since Heidenhain had shown that the application of sodium fluoride to the intestinal mucosa so injured the lining cells of that structure that they no longer assisted in the absorption of materials, a similar effect should be produced on the mesothelial cells. And therefore when no variations in absorption were observed after the use of sodium fluoride they conclude that the mesothelial cells do not exert any activity on the removal of solutions from the serous cavities. They do not offer any explanation of how the final, balanced, isotonic solution is absorbed except to note that it may take place by way of the lymphatic vessels. The suggestion made by Leathes and Starling that isotonic solutions are absorbed via the lymphatics, has been strongly substantiated by the observations of Bolton (8). He studied the question of absorption with especial reference to the routes involved and, in addition to finding that other lymphatics than those of the diaphragm are involved, he also comes to the conclusion that the absorption of isotonic solutions, serum, etc., takes place by way of the lymphatic route. He also points out that even in a solution which is not isotonically balanced only the finest particles are absorbed via the blood vessels.

Fleisher and Loeb (47, (48), (49), (50), (51), (52), (53), (54), as part of an elaborate study on body fluids in general, investigated the absorption of substances from the peritoneal cavity by changing the osmotic pressure of the circulating blood through the administration of adrenalin, caffein, etc. They found that any substance which increased the osmotic pressure of the blood brought about coincidentally an increased absorption from the peritoneal cavity. From these findings they conclude that the mechanism of absorption from the peritoneal cavity is in large measure dependent upon variations in the osmotic pressure of the blood. Cohnstein (23) suggests that when solutions are introduced into the peritoneal cavity an osmotic equilibrium is established between the blood stream and the peritoneal fluid. The absorption which takes place after this equilibrium has been reached is due to the presence of a small amount of colloidal material in the

blood stream which cannot pass into the peritoneal cavity and hence exerts a continued osmotic pressure upon the substances in the peritoneal cavity. This explanation has not seemed satisfactory to a number of observers owing to the fact that blood serum is also easily absorbed. Kjöllerfeldt (81) studied the absorption of albumins, proteoses, peptones, and amino-acids, from the peritoneal cavity. He found that the absorption of amino-acids was much more rapid than was that of the more complex bodies and so concluded that the lining cells of the peritoneum and the endothelium of the blood vessels possess a differential permeability with regard to these substances. This differential permeability, however, he distinguishes sharply from the specific activity which is present in the lining epithelium of the intestine. And he thinks that the differences between his results on the absorption of various protein decomposition products from the peritoneal cavity and those obtained, by Messerli and others, in similar experiments on intestinal epithelium, can be explained on the basis of a differential permeability in the peritoneum and specific cellular activity in the intestinal epithelium.

In connection with his studies on the hydrophilic colloids Martin H. Fischer (46) has explained the absorption of fluids from the peritoneal cavity in terms of his general theory. He believes that the tissues composing the peritoneum are constantly unsaturated with water and that water is therefore taken up from the peritoneal cavity by these tissues. He further believes that the presence of carbonic acid in the circulating blood brings about a transference of the fluid from the peritoneal tissues to the blood stream, thus reducing the saturation of the latter and permitting more fluid to be absorbed from the peritoneal cavity.

Recently three reports have appeared on the subject of absorption from the peritoneal cavity, in which, as the result of very careful and accurate measurements, the observers are forced to the conclusion that there is some other mechanism involved in this absorption than merely the processes of osmosis and diffusion. One of these investigators, Hara, concludes that this activity is inherent in the mesothelial cells themselves. Clark (20), though basing his study of peritoneal absorption upon the principles of osmosis and diffusion, states that

> no attempt is made to explain the absorption of fluids which are in complete equilibrium with the blood such as serum, but the absorption of such fluids is comparatively slow and any absorption due to these unknown causes would be

masked in the experiments described by the more rapid changes due to the differences due to the rate of diffusion.

Hara (64) has definitely concluded that the mesothelial cells are active in absorption from the peritoneal cavity. He bases his conclusion upon the rate with which fluorescein passes from the peritoneal cavity into the blood stream under various conditions. This ingenious method is based upon the fact that when this substance is present in the blood stream even in minute quantities it can be readily detected in the anterior chamber of the eye by direct observation. By using this method and by varying the conditions in the peritoneal cavity, Hara has demonstrated that in many cases the changes in the time required for the fluorescein to enter the blood stream could only be explained by some peculiar change in the lining cells themselves. For example he found that when fluorescein was introduced into the peritoneal cavity, immediately after the removal of a large amount of blood, it could not be detected in the eye at all. He points out that it would therefore seem that the diffusion of this substance into the blood stream would rather have been accelerated under such circumstances unless the lining cells had interposed a barrier to this increase. Again he found that when fluorescein was administered 4 to 6 minutes after the introduction of water into the peritoneal cavity it required three minutes and thirty-eight seconds for it to appear in the eye. But if he waited half an hour after the introduction of the water the time required for the appearance of the fluorescein was one minute and forty-eight seconds. This great discrepancy Hara explains as due to a temporary change in, or injury to, the mesothelial cells as the result of the imbibition of water which alters their specific physiological activities. Finally Putnam (115) has studied the question from the standpoint of the passage of substances from the blood stream into the peritoneal cavity, but incidental to this investigation he also performed experiments on the absorption of substances from the peritoneal cavity into the blood stream. In summarizing his findings he indicates that they are not wholly in accord with purely osmotic principles, leaving certain factors as yet to be explained. He says:

Changes in volume of hypo- and hypertonic solutions in the peritoneal cavity occur in the direction which might be predicted from a consideration of the osmotic forces at work, but never to exactly the extent demanded by the theoretical laws of pure osmosis. Absorption into the blood-stream is always greater than the expected diffusion from the peritoneal cavity, and sometimes very much greater.

The conclusion is drawn that while the major part of the transfer of crystalloids to and from the peritoneal cavity may be explained as being due to a simple colloidal membrane, the presence of another pathway, capable of transmitting colloids and particles as well as true solutions, and oriented only away from the peritoneal sac, must be predicated. Whether this second mode of transmission is due to some form of "vital activity" (phagocytosis, potocytosis), or whether it is due to simple mechanical filtration through intercellular spaces, actual or potential, of appropriate size, is still obscure.

Thus it seems indicated from the more recent and more exact work, that there is a small part of the absorption which takes place from the serous cavities that cannot be explained on the basis of the known physical laws of osmosis and diffusion. There seem to be two possible explanations of the mechanism involved in this absorption of isotonic solutions. The first of these is that the active force is in some way bound up in the metabolic activities of the mesothelial cells and is therefore controlled by laws similar to those active in other cellular functions. That this activity is less in amount than the activity found in the lining cells of the intestine, or in the secreting cells of such organs as the pancreas, may be related, at least in part, to the differences in the cytoplasmic extent of these different types of cells. The second possible explanation of the absorption from the peritoneal cavity of isotonic solutions is that the force active is mechanical, i.e., abdominal pressure, respiratory movements, etc., and that the isotonic solution is gradually forced into the lymphatics or blood vessels by pressure. It does not seem probable that this part of the absorptive activity of the peritoneum can be due simply to the pressure of mechanical openings (as suggested by Putnam) since there is no evidence of any equalization in types of colloidal content other than what can be explained on the basis of diffusion through the walls of blood vessels.

Clinical considerations. A great many investigations have been carried out with the object in view of determining the relationship of the serous membranes to certain practical applications of various therapeutic measures. Among these we must also include considerations in regard to the formation of, and the prevention of adhesions, the prevention of the absorption of bacteria, toxins, etc., and the utilization of absorption from the peritoneal cavity as a mechanism for the administration of whole blood.

On the basis of the work of Muscatello and von Recklinghausen, i.e., the finding that the diaphragm is the principal route of absorption, Clark (21), (139) suggested the use of a posture, as a prophylactic to prevent postoperative peritonitis, so arranged that the diaphragm

would be the lowest part of the peritoneal cavity and thus allow the greatest possible absorption of materials from the peritoneal cavity. This posture was, however, not generally used because it soon became apparent that the increased toxemia was too great a disadvantage to the patient. This was expressed by Fowler (57), (58) when he advocated the use of the opposite position, i.e., sitting posture, in order to prevent absorption from the diaphragm. This question of position after abdominal operations and in the treatment of peritonitis has been commented upon from a clinical standpoint by numerous observers, and was finally subjected to a careful analysis by Dandy and Rowntree (35), (36), who demonstrated that the absorption from the peritoneum was approximately the same in all positions except when the animal was suspended by the front legs, in this position they found there was 15 per cent less absorption than in any of the others.

Blackfan and Maxcy (7) utilized the peritoneal route for introducing solutions of sugar and sodium chloride in treating infants in whom it was difficult to do venepuncture. They found no evidence that there was any especial danger from this procedure as long as the proper precautions were taken to prevent infection and injuries to the viscera. The infants treated in this manner responded equally as well as those in whom the saline or dextrose was administered intravenously. Cunningham (25) studied the effect of 5 per cent dextrose solution, injected daily, into the peritoneal cavities of a group of rats, over a period of two weeks. He found that there was a definite change in the mesothelial cells, which rounded up and became cuboidal, but that, in spite of this, the solution was as rapidly absorbed after two weeks as on the first administration. He also noted a definite gain in weight of all the animals and did not find any infections, adhesions or injuries to the viscera resulting from this procedure.

The use of the peritoneal route for the administration of whole blood has often been suggested. Many observers have transfused blood into the peritoneal cavity and all have agreed that it disappeared with considerable rapidity. Among the various factors involved in this procedure the problem of the clotting of blood in the pleural and peritoneal cavities has received the most attention. There has been much argument as to whether the serous membranes prevent clotting, the principal evidence in favor of this view being that when these cavities were aspirated several hours after the introduction of blood, unclotted blood could be removed. Delrez (38) has recently restudied this problem carefully and has found that when the cavities are opened there

are clots in their recesses; showing that some clotting had taken place. In regard to the practical utilization of the intraperitoneal route for the administration of blood Siperstein and Sansby (129) have recently reworked the problem both experimentally and in patients. They consider that it is a safe procedure in animals and have also used it successfully in the human. They found that a rabbit can absorb about one-fifth of its own blood volume in four hours, and that intraperitoneal transfusion produced an immediate increase in blood values which, however, were somewhat temporary during the time of absorption, but at a later time there was a definite and permanent increase in the number of cells and in the per cent of hemoglobin. They conclude that there is a true absorption of blood corpuscles as such, and not merely an absorption of fluid from the injected blood or of decomposition products from the blood itself. It seems possible, therefore, that the peritoneal cavity does offer an available route for the administration of blood to patients in whom the intravenous route is for any reason out of the question, but in such a procedure every precaution should be used to avoid infection and injury to the viscera.

The prevention of adhesions has also been studied by numerous observers and various methods have been suggested for the prevention of their formation. Surgeons have emphasized the necessity of covering denuded areas with peritoneal grafts, and the uniformly good results obtained from such careful repair is known to every surgeon. But when the areas are inaccessible or otherwise hard to handle the problem becomes more difficult. Other methods which have been proposed for the prevention of adhesions are the use of the cautery, (Keltenborn (79) and Webster 140)) and keeping the viscera from the denuded area by means of gravity (Tinker (134) and Clark (22)). The introduction of various types of foreign materials, including gases (oxygen), drugs (adrenalin, pilocarpine, thiosinamin), oils (lanolin, vaselin, olive oil, mineral oil, castor oil), iodoform, agar, etc., have been tried and all have proven either useless or harmful. Hertzler (71) has expressed the truth concerning the prevention of adhesions in an excellent way:

> The rule is universal that what the peritoneum cannot absorb it walls off or dies in the attempt. Any foreign body not capable of prompt absorption is encapsulated. Therefore any foreign body placed in the peritoneal cavity to prevent adhesions most certainly induces that which it was intended to prevent.
>
> All that the surgeon can do to prevent adhesions is to operate with the utmost gentleness, placing the minimum of foreign bodies of all sorts, either temporary or permanent, within the abdominal cavity, and cover every denuded surface as carefully as though it were an unpardonable sin.

Finally there have been a number of experiments performed with the object in view of increasing the resistance of the peritoneum to bacteria and toxins or of preventing the absorption of these injurious agents. These experiments have so far yielded very little information of any importance but nevertheless they are essentially in the right direction. Among the more interesting of these studies are those of Kawasaye, Hoehne, Reschke, Douglas, Gay and Morrison, and Narat. Hoehne (75), (76) attempted to retard the absorption of bacteria and toxins from the peritoneal cavity by the irritation of the lining membranes. He found that if the bacteria (using streptococci and colon bacilli) were introduced simultaneously with the irritating agent, the resistance of the animals was lower than in controls, while in those whose peritoneums had been irritated several days previous to the introduction of the infectious agent, the resistance was decidedly increased. The results of these experiments together with the findings in patients led Hoehne to conclude that stimulation of the peritoneal membranes offers protection against infection only by the localization of the bacteria in situ, and is purely a mechanical affair due to a retarding of absorption by the formation of a thin layer of fibrinous material over the surface of the peritoneal membranes. He does not believe however, that any protection is afforded against the absorption of toxins.

Among the modern methods which have proven of clinical value, one of the most noteworthy, has been the administration of hypertonic solutions. Hypertonic solutions of sodium chloride have been used following hemorrhages by von der Velden (137); to diminish the size of the brain and so decrease intracranial pressure by Weed and McKibben (142), (143), Cushing and Foley (34) and Sachs and Belcher (124), and for a variety of other purposes by Wilder and Sansum (145) Litchfield (89), (90) Erlanger and Woodyatt (44) and others. This method has been applied to the prevention and treatment of peritonitis by Kuhn (84), Reschke (118) and Narat (106). These observers have agreed in general that the administration of hypertonic solutions increases the resistance of experimental animals. Reschke studied the effect of sugar and salt sprinkled on the viscera and concludes that the former is better than the latter, both because it takes longer for the concentrated sugar solution to reach isotonicity and because it has more "injurious effect" on the bacteria. Narat has recently studied the introduction of a twenty per cent solution of glucose and finds that the results in animal experimentation are sufficiently en-

couraging to warrant advising its use in treating peritonitis in the human. Since the absorption of toxins is the most important factor with which we have to deal in peritonitis Douglas (39) suggests the use of adrenalin to reduce or prevent this taking place. He finds that when adrenalin is given intraperitoneally the absorption of any soluble substance is greatly reduced and explains this as due to vasoconstriction. These findings are in line with his observations on the absorption of decomposition products from the tissues which are produced following burns. Finally Gay and Morrison (59) have studied the rôle of the clasmatocytes of the peritoneal exudates in streptococcus infections and have found that these cells are "implicated in both the active and passive forms of acquired streptococcus immunity." They found that the injections of a plain infusion broth caused the formation of an exudate rich in clasmatocytes in contrast to the polymorphonuclear leucocytes called out by aleuronat, egg albumin, etc. Using this method to produce an exudate rich in clasmatocytes they demonstrated that animals in which these cells had been called out in large numbers were far more resistant to streptococcus infections than normal animals or those with an exudate rich in polymorphonuclear leucocytes. To what extent this method is capable of chemical application is yet to be determined.

Altogether then it seems that there are several possible means whereby peritoneal infections may be to some extent controlled, but none of them have been very widely used clinically as yet, and none of them are wholly without disadvantages. It is therefore quite obvious, both that these methods should be subjected to further careful study, and that search should be made for other methods capable of modifying the peritoneal membranes, so that greater resistance to infection can be obtained.

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VENOUS PRESSURE AND ITS CLINICAL APPLICATIONS

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THE PHYSIOLOGICAL MECHANISMS DETERMINING VENOUS PRESSURE. In the vascular system with the heart at rest, the pressure of the blood is uniform throughout. The mean "hydrostatic pressure" (1) measures in the dog approximately 10 mm. Hg. When the heart is beating, blood is taken from the veins and forced into the arteries, and differences in pressure develop due to the resistance offered to the moving stream. In the blood vascular system the physical conditions determine that the pressure must be highest at the driving force or head of pressure, the arterial outlet from the heart, and must continually fall to the end of the system, the venous inflow into the heart. According to most writers, the resistance is mainly concentrated, and hence the fall of pressure is most pronounced in the smaller vessels of the periphery, the arterioles, capillaries and venules (for a contrary view see Bergolomez (2)). Physiologists came therefore to speak of the "peripheral resistance," and to use it to explain the marked fall of blood pressure occurring between the arterial and venous portions of the vascular system. To the older physiologists, whose views were largely deductions from the physical conditions present and from studies of artificial schemata of the circulation, the problem of the cause of venous pressure and its variations was a relatively simple one. It was believed that the action of the heart does not increase the mean pressure of the system, but merely alters its distribution; the mean hydrokinetic and hydrostatic pressures are identical. The two obvious factors concerned, the head of pressure represented by the cardiac output, and the variable peripheral resistance, were used to explain variations in the pressures in the arterial and venous sides of the system; and these with the addition of possible changes in blood volume served for the well known laws of Marey (3).

The peripheral vascular bed. When venous pressures were measured in the living animal under varying conditions, it was found that they by no means always followed the formulated rule. Bayliss and Starling (4)

showed that abolition of vasoconstrictor control by section of the cord or splanchnic nerves, causes a marked fall in arterial pressure but little or no change in venous pressure, and they proposed another factor influencing venous pressure, a peripheral vascular bed variable in capacity through wide limits. Removal of vasoconstrictor tonus, by greatly lessening peripheral resistance, should cause a marked rise of venous pressure, but this is compensated for by an increase in the total vascular bed. Increased peripheral resistance from splanchnic stimulation causes, instead of a fall, a slight rise of venous pressure, explicable on the assumption of a reduced vascular bed. The effect of variations in capacity of the vascular bed is to change the "mean pressure" of the whole system, and this compensates for changes that would otherwise occur due to alteration in peripheral resistance. The rise of venous pressure on vagal inhibition, usually explained by reduction of cardiac activity, was referred mainly to a reduction in the total vascular bed due to a widespread constriction, the result of medullary anemia and stimulation of the vasoconstrictor center. The proposal of Bayliss and Starling was the beginning of numerous contributions to the subject, in the main tending to establish this factor as one of essential importance, but not devoid of efforts to explain the experimental results on other grounds (5). The literature is large and no attempt will be made to present a complete bibliography, since this has been done up to 1920 in a review by Connet (6). The early work of Roy and Brown (7) had given a suggestion as to possible alterations in the vascular bed, by offering evidence for a variable tonus in individual capillaries and venules. Numerous subsequent workers have contributed evidence pointing to the same conclusion. Krogh (8) has demonstrated the marked difference of the capillary circulation in the muscle during rest and activity, and has shown the inadequacy of the older explanation by which this was accounted for by variations in arterial tension. In rest many capillaries are occluded, to become patent during activity. Many subsequent observations have presented evidence tending to demonstrate the relative independence of venous pressure and peripheral resistance. Nolf and Plumier (9) found little effect on the venous pressure in the systemic veins during the enormous vasoconstriction present in asphyxia until the heart began to fail. Roy and Sherrington (10) observed a fall of venous pressure in asphyxia. The venous pressure rises along with the arterial pressure in man with increased carbon dioxide in the respired air (11). Delezenne (12) found femoral venous pressure rising and falling with arterial pressure during Traube vasomotor waves. In the renal

vein the pressure changes were opposite. Similar opposite variations in venous blood pressure in the femoral and renal veins were seen in asphyxia and on cooling the skin surface. Plumier (13) found little influence on venous pressure of reflex vasomotor changes unless cardiac activity was altered. Intravenous injection of the nitrites caused a rise of venous pressure in the jugular while the pressure in the inferior vena cava was unaffected. Hooker (14) noted that both arterial and venous blood pressure fall during sleep. He also found that local changes in vascular tonus produced by warming or cooling the hands, caused no significant change in venous pressure, although the volume of the vein and its size was markedly affected. This observation has been confirmed by White (15). Briscoe (16) found in the "Raynaud Phenomenon" (local stagnation of hand circulation) that there was only very slight or no change in the pressure in the medium size veins, while the skin blanching pressure, interpreted by her to mean capillary pressure, was very high. This phenomenon was interpreted as due to an intense constriction of the small veins, producing a high capillary pressure and stasis. In extreme cases the veins may become very small, indeed, almost invisible, but with unchanged venous pressure. Venous pressure in the internal saphenous vein of the leg in the prone position is usually lower (17), or at least no higher (18) than in the median vein of the arm, while the systolic arterial pressure is from 20 to 40 mm. of mercury higher in the femoral than in the brachial artery (19). The mechanism by which the independent control of tonus in the peripheral vessels is brought about has also been the subject of numerous investigations. This phase of the subject has been comprehensively reviewed by Hooker (20) and by Ebbecke (21). It seems probable that the tonus of the capillaries and venules is both direct, possibly mainly through the carbon dioxide content of the blood (22), (23), and through a venomotor system of nerves. Hooker (24) demonstrated a rise of venous pressure in an intestinal loop preparation as a result of nerve stimulation and at a time when the arterial pressure was zero. The same result was also obtained by reflex stimulation and by direct stimulation of the central nervous system (asphyxia). Whether the contraction was in the larger veins, venules or capillaries, was not determined. That the nervous control extends at least in part to the larger veins seems probable from the work of a number of investigators. For reviews of the literature concerning neuromotor control of the radicals of the portal system, references may be made to the writings of Burton-Opitz (25) and Edmunds (26). That the vagus has an influence upon

the tonus of the superior vena cava seems probable from the work of Knoll (27). Thompson (28) observed segmental constrictions in the superficial veins of the hind limbs on stimulation of the sciatic nerve. This observation was confirmed by Bancroft (29) who traced the nerve path and identified the prevertebral ganglia concerned by Langley's nicotine method. Horiuchi (30) found that the sciatic nerve supplies both constrictor and dilator fibers to the cutaneous veins of the hind limb of dogs, and that they are susceptible to reflex stimulation. Several observers have described the veno-constricting influence of adrenalin, either applied directly to ring preparations of the larger veins (31), (32), or on intravenous injection in the intact animal (33), (6). The control of the peripheral bed is probably in part the result of changes in pressure within the arteries and veins. The depressor reflex, by means of which reduction in the vasoconstrictor tonus (34) and cardiac slowing (35) occurs when the blood pressure rises in the aorta, has long been recognized. Recently McDowall (36) has presented evidence to show that a pressor reflex may arise from the vena cava. A low pressure in the vena cava appears to cause an increased tonus of the vasoconstrictor center as a result of impulses passing up the vagus nerve.

Certain additional and in part more recent work supporting the hypothesis of a vascular bed, variable in capacity through wide limits, should be mentioned. The work of Dale and Richards (37) and Dale and Laidlaw (38) with histamine, proved the enormous influence of vascular paralysis, and furnished a rational basis for the widely prevalent conception of vascular shock as the result of stagnation of blood in a greatly enlarged peripheral bed. Hooker (39) has presented visual evidence of wide variation in the filling of vascular beds under nervous and chemical influences. Meek and Eyster (40), (41) and Eyster and Middleton (42) have demonstrated the adaptability of the circulatory bed and the maintenance of a constant venous pressure following moderate changes in blood volume in the dog and in man.

The main function of this mechanism is probably in reference to the regulation of the volume and pressure of the venous return to the heart. Krogh (43) has called attention to the liver, with its double set of resistance, as probably of particular importance as a variable blood reservoir feeding the vena cava.

Relation of venous pressure to cardiac output. It was early recognized that venous pressure may be influenced by the rate at which the heart received blood from the veins and forced it into the arterial system. A considerable reduction in cardiac output, as for example that occurring

in vagal inhibition, would obviously tend to produce stagnation in the venae cavae and a rising venous pressure. Increased cardiac output, on the other hand, would tend to cause a depletion of the veins and a low venous pressure. In this conception it is clear that cardiac output is the variable and changes in venous pressure the result. A different conception of the relation between cardiac output and venous pressure has developed in recent years, by the recognition of venous pressure as probably the main factor in determining the extent of cardiac activity. Instead of a more or less antagonistic influence, the view has been growing in favor that the two factors are interdependent, and that cardiac activity, since it is regulated by venous pressure, is a most important factor in tending to stabilize venous pressure at an approximate level. The influence of venous pressure seems to be in part on heart rate, ascribed by Bainbridge (44) to a reflex through the vagus nerves. DeGraff and Sands (45) have recently questioned the importance of the increased heart rate produced by a rise of venous pressure, since it occurred in only half of their experiments. They also found after section of both vagi, that an increase in heart rate occurred in one-third of the animals tested when venous pressure was increased, and therefore fail to support the conclusion of Bainbridge as to the mechanism of the increase. The influence of change in stroke volume as a result of changes in venous pressure would seem to be of greater importance. The question as to whether stroke volume is altered by venous pressure and if so, how much, has been a matter of controversy in recent years, and it is only very recently that it would seem a fair agreement is being reached. A presentation of the controversial aspects of this matter is beyond the scope of this paper. A statement of the two divergent hypotheses, reference to recent writings where the evidence on both sides is critically reviewed, and a consideration of a few of the leading or of recent contributions to this question must suffice. According to Henderson and his co-workers, moderate variations of venous pressure, such as probably represent the normal range, produce no tendency to alter the stroke volume of the heart, and variations in output can occur only through changes of heart rate. This has been called the law of "uniformity of cardiac behavior" and the evidence in relation to it has been critically reviewed by Henderson (46). The actual venous pressure which serves to fill the heart during diastole, is obviously the pressure measured within the right auricle (with a manometer balanced against atmospheric pressure), plus the negative pressure of the thoracic cavity. This has been aptly designated as the

"effective venous pressure" (47). According to Henderson, the law of uniformity of cardiac behavior holds with an effective venous pressure of 50 mm. of water or more, since under these circumstances cardiac filling is complete. With venous pressures above the critical level of 50 mm., stroke volume is uniform, and variations in cardiac output can result only from changes in rate. Patterson and Starling (48) and Straub (49), working on a simplified circulation (heart lung preparation), found a direct relation between venous pressure, diastolic filling and systolic discharge up to a maximum venous pressure of 250 mm. of water. The former investigators proposed the "law of the heart," by which systolic output is made a linear function of venous pressure through its influence on the diastolic length of the muscle fiber, and this may be regarded as the extreme antithesis to the law of "uniformity of cardiac behavior." The more recent expressions of opinion seem to indicate a somewhat mid position between these extreme views (50). The work of Patterson and Starling and of Straub is open to the serious criticism of the abnormal condition of the heart, in the absence of possible restraining influence of the pericardium, and the removal of possible influences on tonus exerted through the extrinsic cardiac nerves. On the other hand, a great deal of experimental observation is difficult or impossible to accord with the conception of a uniform stroke volume. The marked increase in cardiac output in exercise, determined by gasometric methods in man (Plesch (51), Krogh and Lindhard (52), Lundsgaard (53), and others) is exceedingly difficult to explain without the assumption of a variable stroke volume. Wiggers and his co-workers have attacked the problem in a series of studies in which accurate intracardiac pressure measurements were made with optical manometers, and obtained evidence for a variable systolic discharge up to a venous pressure just short of beginning signs of cardiac decompensation (250 to 310 mm. of water). The increased systolic discharge is due to increased velocity of ejection and prolongation of systole (54). Meek and Eyster (41), using an x-ray method for measuring cardiac output in the intact animal and varying venous pressure by saline infusion, found a variable stroke volume up to a level of venous pressure of at least 150 mm. of water.

In a recent study of cardiac output in man, based on a new modification of the gasometric method, Henderson and Haggard (55) revised their former figures relating to the resting stroke volume and its change during activity. They found the normal stroke volume to lie between 1.3 to 1.8 ml. per kilo of body weight, maintained without much variation during slight muscular activity but rising to a maximum of 3.5 ml.

during vigorous exercise. It would seem therefore that at the present time the evidence pointing to a variable stroke volume depending upon the venous return is accepted by all workers in this field, and that the only difference of view that may still persist is in regard to the extent of this correspondence.

Effect of respiratory activity. The negative pressure in the thorax facilitates the flow of blood in the vena cava and determines a higher feeding pressure to the heart ("effective venous pressure") than exists outside. The intrathoracic venous pressure (as measured by balancing against atmospheric pressure) is slightly negative, but rises to a definite positive value when the thorax is opened, and this causes a general rise of venous pressure extending into the smaller veins (56). The varying negative pressures in the thorax, associated with the different phases of respiration, produces an evident influence on pressure and flow in the larger veins. According to most workers, venous pressure falls in the experimental animal during inspiration and rises during expiration (57), (56). Mosso (58) found differences in this respect between the venous pressure in the upper and lower limbs, the inspiratory flow and consequent fall of pressure failing in the latter because of the descent of the diaphragm and rise of intra-abdominal pressure. Wortheimer (59) was unable however to confirm this, and found both pressures fall in inspiration. Burton-Opitz (56) observed no effect upon the course of femoral venous pressure on opening the abdomen. The respiratory variations in venous pressure disappear in the smaller veins of the periphery (57). The venous pressure variations with respiration appear to be the result of changes in intrathoracic pressure, and not to be due to the transmission of variations in arterial pressure. They are at least independent of the inspiratory acceleration of the heart (60). The relation of venous pressure to the phases of respiration in man have been studied by numerous investigators, but there is marked lack of agreement. Sewall (61) found in most cases a rise of pressure and an increased filling of the peripheral veins during inspiration, which he explained by an increased inflow from the capillary bed. Fuchs (62) observed a difference between the arm and leg veins, thus confirming Mosso's observation in the experimental animal, and ascribing it likewise to the influence of abdominal pressure. He found a falling venous pressure in the arm vein with inspiration, while the opposite relation held in the leg. Respiratory variations amounted in a few cases to as much as 30 mm. of water. Kroetz (63) noted that respiratory variations in pressure in the peripheral veins of man are often absent, but

usually variations of 0.5 to 2 mm. and often 4 mm. of water were observed. He called attention to the fact that by far the larger part of the variations within the thorax, amounting to approximately 70 mm. of water under average conditions, are absorbed by the inertia of the blood column between the central and peripheral veins. In maximal breathing in a strong muscled subject, variations of 5 to 8 mm. were noted, and this may rise to 10 or 25 mm. in dyspnea associated with thoracic deformation (emphysema and kypho-scoliosis). He did not relate the fall of venous pressure to the phase of respiration. According to Bedford and Wright (64) respiratory variations are not normally present in the veins of the arm. Runge (18) also found them usually absent, but observed a slight fall in venous pressure in inspiration in about a third of all cases, seldom amounting to more than 10 mm. of water. All of the above observations on man, with the exception of Sewall's, were made by direct cannulation of the vein. Waud (65), using an indirect method of measurement, found that venous pressure in the peripheral veins of man rises shortly before or coincident with expiration and continues to rise into the phase of inspiration.

The effect of over-ventilation introduces, in addition to the mechanical factor of intrathoracic pressure, the possibility of another factor, namely, changes in the gaseous content of the blood. Henderson, Prince and Haggard (66) found a fall of peripheral venous pressure amounting to 80 to 110 mm. of water in voluntary over-ventilation in six normal subjects, ascribed by them to reduction of the carbon dioxide content of the blood. Bedford and Wright (64) found a fall of only 10 to 30 mm. and Kroetz (63) of only 7 to 25 mm. under similar circumstances. According to the last observer, the fall is complete within the first twenty seconds of the period of hyperpnea. The influence of abnormal changes in intrathoracic pressure has been observed particularly in the Valsalva experiment (expiratory effort with the chest in inspiratory position and glottis closed), and in the Müller experiment (inspiratory effort with the chest in expiratory position and glottis closed). The former will obviously produce a rise of intrathoracic pressure with the ultimate attainment of a high positive value, the latter a marked fall in intrathoracic pressure to a low negative value. Moritz and v. Tabora (67) found that the venous pressure may rise to 420 mm. in the Valsalva experiment. Burger (68) found the peripheral venous pressure rising as high as 500 mm. in the Valsalva and falling to 40 or 50 mm. of water in the Müller experiment (from a normal of 100 mm.). Kroetz (63) observed values of 400 and 25 mm. of water under these conditions.

Possible influences of abnormal intrathoracic pressure, due to malformations of the thorax or pulmonary diseases, will be found under the clinical section of this paper. Respiration probably also exerts an influence upon peripheral systemic venous pressure in offering a varying resistance to pulmonary flow. Burton-Opitz (69) found that inflation of the lung to a point equal to that occurring in normal inspiration is associated with the largest blood flow and lowest pressure in the inferior vena cava. Delezenne (70) found a rise of venous pressure in the renal vein on excessive artificial ventilation in curarized animals, explained by compression of the pulmonary capillaries and decrease of pulmonary flow. The right ventricle works against an increased resistance and systemic venous pressure rises.

Aspirating effect of cardiac systole. The old conception that the ventricles act as a suction as well as a force pump has been generally abandoned (71), but there is an aspirating effect upon blood in the vena cava during ventricular systole, probably the result of direct traction upon the walls of the auricles by the descent of the ventricular base (72). It is this which produces the negative phase of the venous pulse and the systolic collapse of the jugular veins. Variations in venous flow in the veins near the thorax, associated with the different phases of the cardiac cycle (venous pulse), are no longer detectable in the peripheral veins, but obviously this aspirating effect on the auricle must exert some influence upon mean venous pressure throughout the system, and probably in a degree proportionate to the extent of ventricular systole.

Effect of gravity. While the hydrostatic factor has to be taken into consideration in all parts of the vascular system above or below heart level, the greatest influence is exerted upon the veins, because of the relatively low pressure in these vessels. The hydrostatic factor amounts to approximately 0.76 mm. of mercury pressure for each vertical centimeter. In the vessels of the foot, in the average man in the erect position, this would amount to about 85 mm. of mercury. Hill (73) and others have shown, that if the tonus of the peripheral vessels is destroyed, the vascular bed becomes engorged with blood and death occurs from decreased return of venous blood to the heart. The normal tonus of the vessels supplied by the splanchnic nerves appears to be especially important in this connection, and to be developed to its greatest degree in upright animals (monkeys, man). Hill found that placing a dog in an upright position with hind feet down, caused a definite rise of pressure in the portal vein and a fall of pressure in the cerebral veins (torcular). The influence of gravity on venous pressure

in man has been studied by v. Recklinghausen (74). Venous pressure rises in the veins of the hand held at different levels below the heart until sufficiently high to overcome the hydrostatic pressure and ensure a return of venous blood. If the hand is lowered quickly, the rise occurs slowly as the veins fill from the capillaries. Sewall (61) found a rise of pressure in an arm vein when the other arm was elevated above the head, presumably due to the hydrostatic emptying of the veins into the vena cava. In the veins of the foot, in the sitting or standing position, v. Recklinghausen (74) found that the venous pressure did not rise sufficiently to overcome the hydrostatic pressure. Deficits of from 7 to 58 cm. of water (5 to 43 mm. of mercury) were found. The actual pressure usually attained was about equal to the hydrostatic level of the entrance of the veins into the pelvis.

Contraction of skeletal muscles. Under normal conditions of activity in the erect or sitting position, the return of venous blood from the lower extremities is facilitated or indeed made possible by contraction of the skeletal muscles compressing the veins. Return of the blood distally after the contraction, is prevented by the disposition of the venous valves. (For review of this subject see Ledderhose (75).)

THE NORMAL VENOUS PRESSURE. METHODS AND RESULTS. Venous pressure was first measured in the classical experiment of Stephen Hale (76), by the simple expedient of connecting an upright tube to a vein of a living animal and measuring the height to which the blood rose. Using modern laboratory methods, Jacobson (57) found values in the sheep ranging from +11.4 mm. in the crural to −0.6 mm. of mercury in the left subclavian vein. Burton-Opitz (56) obtained similar figures in the dog, and stated that the fall in pressure amounts to about 1 mm. of mercury for each 35 mm. in distance from the periphery toward the heart. The pressure in the same vein in different animals approximated closely, and as compared with arterial pressures, fluctuations in the venous system were slight. Negative pressures were first encountered in veins close to the thorax. Bergolomez (2) measured the pressure directly from veins as small as 0.2 to 0.3 mm. in diameter in rabbits (small ear veins) by the use of needle cannulae, and found considerable variations in different animals (4 to 23 mm. of mercury).

Measurement of venous pressure by direct cannulation of peripheral veins in man has been done by a number of workers. Moritz and v. Tabora (67) found the pressure in the median vein of the arm to vary from 10 to 90 mm. of water (0.8 to 7 mm. of mercury). Schott (77)

found an average figure of 70 mm. of water by this method in twelve normal subjects, with variations between 50 and 130 mm. Claude, Porox and Rouillard (78) gave figures of from 80 to 240 mm. of water in a group of unclassified patients. Fuchs (62) obtained a mean of 76 mm. of water on fifty normal subjects, with variations between 15 and 125 mm. LaConte and Yacoel (79) and Villaret, St. Girons and Grellity-Bosviel (80) establish somewhat higher normal figures, 130 to 140 mm. of water in men and 120 mm. in women. These figures have been confirmed by Gazzotti (81) Castellotti (82), on the other hand, using similar technique, established a normal range between 40 to 70 mm. of water in adults, independent of sex or age. The figures were somewhat lower in children (eight or under, 20 to 30 mm.). Bedford and Wright (64) found considerable variation in thirty-five normal subjects. Venous pressure was between 50 and 100 mm. of water in 56 per cent and between 50 and 150 mm. in 90 per cent. Most of the preceding figures were obtained in the dorsal prone position, with the vein at approximate heart level or corrected to this position.

Most of the studies that have been made on the venous pressure in the peripheral veins of man have been carried out with some form of the indirect method, first employed by Frey (83), in which pressure is made over a superficial vein and the amount required to produce collapse of the vein determined. Frey used a pelotte, moveable in a vertical plane, to which weights were added until collapse of the underlying vein occurred. He found the pressure in normal subjects remarkably constant. v. Basch (84) used a glass chamber placed on the skin over the vein and connected to a water manometer and pump. Air was forced in until the vein was seen to collapse. He found the pressure in a superficial hand vein to average 10.7 mm. of mercury (144 mm. of water) in seventeen normal subjects in the sitting position, with variations in different subjects between 4 and 12.5 mm. (54 to 179 mm. of water). Oliver (85) and Sewall (61) used a pelotte similar to that of Frey, except that a gauge replaced the weights for the determination of the pressure applied. v. Recklinghausen (74) introduced a hollow rubber capsule, open above and below, and connected by a side opening to a pump and manometer. The capsule was placed over the vein and the upper opening covered by a piece of glass. The vein was observed through the glass and air forced in until collapse occurred. He gave a few normal figures in the prone position of from 30 to 50 mm. of water. Hooker and Eyster (86) constructed an apparatus on this principle, in which the rubber capsule was replaced by a metal box, covered below by a rubber

membrane with a central opening, and with sides and top of glass. Adherence of the rubber to the skin surface was effected by painting it with glycerine. Pressure, raised to the point of collapse of the vein, was obtained by squeezing a rubber bulb, and read on a water manometer. Determinations made on ten normal men in the sitting position gave an average of 71 mm. of water and variations between 40 and 90 mm. A number of readings made on two individuals throughout a day varied between 40 and 100 mm. A modified instrument, using an all glass cannula cemented to the skin by celloidin solution, was subsequently developed by Hooker (87). Brown (88) used a glass capsule closed at one end by very thin rubber; the other end communicated with a water manometer and bulb. The membrane of the capsule was distended, the vein milked with the finger to a valve until empty, the capsule applied and the pressure allowed to fall until the vein filled. In the prone position he obtained readings of from 30 to 90 mm. of water. In order to avoid prolonged collapse and possible stagnation in the vein with venous pressure rising above its previous value, White (15) has recently devised an apparatus producing momentary pressures of any desired amount which are applied to the vein through a capsule. The pressures are applied, starting with one sufficiently high to produce collapse, in decrements of 5 mm. of water, until the lowest pressure sufficient to produce a momentary collapse of the vein is found. Determinations made on nine subjects in the sitting position gave readings of from 50 to 95 mm. of water. Frank and Reh (89) developed a method using two sphygmomanometer arm bands, one placed around the upper arm, the other around the forearm. The lower cuff was lightly inflated, and by its connection with a tambour writing on a drum, gave a record of variations in the volume of the forearm. The upper arm band was connected with a water manometer and was inflated slowly until the volume of the forearm began to increase, indicating an interruption of venous flow. The pressure read at this time was taken as the mean venous pressure of the superficial veins of the forearm. In the normal subject values from 10 to 60 mm. of water were found. The same procedure has recently been employed by Payan and Geroud (90). A third method for the estimation of venous pressure in man was proposed by Gaertner (91). It consists in determining carefully the level at which a superficial vein of the hand is seen to go from a state of filling to one of collapse. The hand is first lowered and then raised slowly. Collapse of the vein occurs at the level where the negative hydrostatic pressure equals the positive venous pressure, and if this is compared

with the estimated level of the right auricle, one can express the venous pressure in millimeters of water (blood). The vein represents a manometric tube connected with the right auricle. Gaertner found that collapse occurred in the normal subject in the sitting or standing position when the vein was brought to the level of the sternal insertion of the third to the fifth rib.

Critique of methods. A priori, the direct method of venous pressure determination in man, in which a trocar is inserted directly into the vein and connected with a manometer, would seem to be more accurate than any of the indirect methods. Hooker and Eyster (86) compared the capsule pressure method with the direct method and found a close correspondence. Similar concordance has recently been established by Tatterova and Serf (92) and by Payan and Geroud (90). The most marked divergence in readings in both normal and clinical subjects has been reported by different workers using the direct method.

Moritz and v. Tabora (67), the first to use this method, obtained figures for normal venous pressure which have been approximately confirmed by several more recent workers (Schott (77), Fuchs (62), Castellotti (82)). Another group of investigators, using a somewhat modified technique and a special manometer (Villaret, St. Girons and Grellity-Bosviel (80), LaConte and Yacoel (79), Gazzotti (81), obtain much higher and more constant figures. One of these observers (81) obtained readings varying only between 120 and 140 mm. of water in twenty-nine normal subjects, a constancy unique in the literature of this subject. On the other hand, another group of workers (Bedford and Wright (64)) obtained probably the greatest variation that has been recorded in any normal series; readings between 50 and 200 mm. of water in thirty-four normal subjects. It would seem that there must be something radically wrong in the application of a method when such divergent results are obtained by different investigators. The technical difficulties of this method have been recently considered by Bedford and Wright (64) and by Runge (18). The latter observer mentions the use of a trocar with bore so small as to cause capillarity, faulty placing of the trocar in the vein and occlusion of the connection with the manometer as the more important causes of failure. He made three readings, on equalizing the vein pressure with the manometer in every case, and discarded those in which there was any considerable variation, when a hematoma around the vein occurred, and when a Valsalva experiment failed to show a definite rise in pressure. Measurements were accepted on only forty-eight of seventy-five subjects examined. Any one who has had experi-

ence with the trocar method in animals will appreciate the many possibilities of error, particularly in the absence of respiratory oscillations, a condition which appears to be quite common in man. Another factor that has not been considered, is the probable influence on venous pressure in the arm from the partial or complete occlusion of one of the larger veins (the median) draining the peripheral bed. Many of the determinations made by the direct method are higher than those obtained by workers using indirect methods. A criticism against the usual application of the indirect method is that the collapse of the vein under pressure, by producing stagnation of venous flow, may give an abnormally high reading. The method developed by White (15) and described above was designed to overcome this objection. He obtained readings 10 to 40 mm. lower by this method than by the usual one of forcing air into a capsule by means of a pump. Since the usual procedure in the capsule method is to increase the pressure from zero to the point of collapse, interruption of the venous flow should be only momentary. It would seem obvious that if the flow is interrupted for any considerable period, abnormally high readings would result. It should be noted in this connection, however, that Frank and Reh (89) found by their graphic method, that the rise of volume of the arm on obstructing the venous flow occurs slowly, and would hardly influence the usual venous pressure reading unless the vein is obstructed for an unnecessary length of time. If any of the several indirect methods of determining venous pressure are open to this criticism, it would be the method of Frank and Reh, because of the widespread obstruction to venous flow at the time the readings are made. The indirect method has one great advantage over the direct, in that numerous readings can be made on the same patient without difficulty or discomfort. This is of great value, especially in impending or already present cardiac decompensation, when it is the trend of venous pressure as much as its actual height, which is important. In all but a few of the clinical cases reported by workers using the direct method, only a single venous pressure reading is given.

Influence of sex. Slightly lower average venous pressures (10 to 20 mm. of water) have been noted in women than in men, by Hooker (14), Briscoe (16), LaConte and Yacoel (79), and Villaret, St. Girons and Grellity-Bosviel (94).

Diurnal variation. Hooker (14) found a gradual rise of venous pressure during the waking hours and a gradual fall during sleep. In his own case, the pressure was close to 100 mm. of water before arising in

the morning, and from this point rose irregularly during the day to reach approximately 200 mm. before retiring.

Influence of posture. Barach and Marks (93) studied arterial and venous pressures in twenty-six human subjects who were placed in an erect or horizontal position by an adjustable table, all active effort on the part of the subjects being avoided. Venous pressure varied between 80 and 180 mm. of water in the erect and between 35 and 110 mm. in the horizontal position. The rise of venous pressure on assuming the erect posture was associated with a rise of systolic and a fall of diastolic arterial pressure. Villaret, St. Girons and Grellity-Bosviel (94) noted a rise of venous pressure in the arm vein in the sitting as compared with the prone position. Rises to 250, 280 and 300 mm. were noted in three cases. Arterial pressures fell slightly.

Influence of age. Extensive observations on individuals in age groups between 5 and 85 years have been made by Hooker (87). All readings were made in the sitting position. The figures show a gradual rise with age from an average in the youngest group of 83 mm. to 267 mm. of water in the oldest group. This influence would seem to be in part postural, since Castellotti (82) and Eyster and Middleton (95) observed no difference referable to age in normal subjects in the prone position after rest in bed.

Effect of bed rest. Hooker obtained readings varying from 30 to 150 mm. of water on three patients with normal cardiovascular system at bed rest for surgical conditions (14). Runge (18) found an average of 54 mm. of water with variations between 48 and 66 mm., on six similar patients. Extensive observations on hospital patients in bed for minor surgical conditions or rest treatment were made by Eyster and Middleton (95). The usual readings were close to 50 to 60 mm. of water. The highest single reading obtained in hundreds of observations was 110 mm. It is of importance to determine this range accurately as a normal to apply to the study of cardiac and other cases ill in bed, particularly since, as has been noted by Hooker (87), the usual venous pressure found in cardiac decompensation, approximating 200 mm. is the average for his age group of 45 to 55 years of normals in the sitting position, an age group represented by many cardiac cases who become decompensated. Under rest conditions in the prone position, venous pressure becomes stabilized within fairly narrow limits and appears to be independent of age.

Effect of muscular activity. Sewall (61) found a rise of venous pressure in the hand which was exercised by alternately flexing and extending

the fingers, and explained it as due to increased inflow of blood resulting from local vascular dilatation. With both hands supported at the same level, exercising one had no influence on the venous pressure in the other. More vigorous exercise caused a general rise of venous pressure. Hooker (96) made observations during violent exertion on a stationary bicycle, and found an increase of from 70 to 140 mm. of water in five subjects. The average rise was 100 mm. Schott (77) studied the influence of elevating the leg at an angle of sixty degrees while lying prone, and holding this position as long as possible. Venous pressure, as measured in the median vein by the direct method, showed little or no change in normal subjects, but a distinct elevation occurred in cardiac cases and approximately proportionate to the degree of cardiac involvement. It should be noted that the rise of venous pressure under these conditions may be explained by the emptying of the veins of the elevated leg into the venous cisterns (cf. Sewall (61)). Villaret, St. Girons and Grellity-Bosviel (94) noted a rise of 20 mm. after a prolonged walk. Gazzotti (83) observed a rise of 10 to 30 mm. of water in four subjects after a ten-minute run. Jacquemium (97) found a rise of 10 to 20 mm. of water in the arm vein on raising and lowering the leg several times while in the prone position. Bedford and Wright (64) found venous pressures of from 90 to 195 mm. of water immediately after severe exercise to six subjects, falling rapidly to normal during a rest period. Runge (18) found a rise of 20 mm. of water in an arm vein on rapid flexion and extension of the fingers, while a fall of 30 to 40 mm. was observed in a leg vein when the foot was alternately flexed and extended. White (98) found in light muscular work on a stationary bicycle an elevation of venous pressure amounting to 40 to 50 mm. of water, falling immediately to or below normal on its cessation. With more severe work, the venous pressure at first rose to a greater extent than with light work, but after a short time began to fall and returned to normal or below within a few minutes. When the exercise was stopped there was usually a slight further fall. White and Moore (99) have studied the effect of static and dynamic exercises on venous pressure, and concluded that "the tendency of the increased venous return during exercise to raise the venous pressure is partially or completely compensated for by the venodilator mechanism, the increased aspiratory action of the thorax, the increased heart rate and loss of diastolic heart tone." The extent of the compensation processes varied somewhat in different individuals, and did not always suffice to keep the venous pressure entirely constant.

VENOUS PRESSURE IN DISEASE. *Cardiac decompensation.* This is the

one clinical condition in which the great majority of observers agree that consistent and significant venous pressure changes occur. Frey (83) in 1902 stated that a rise of venous pressure may be an early sign of impending cardiac failure and Gaertner (91) a year later noted venous pressures as high as 230 mm. of water in several cases of decompensation in mitral disease. Hooker and Eyster (86) in 1908 reported several cases of decompensation in some detail, in which venous pressures were at first high, falling to normal as compensation was reëstablished. Moritz and v. Tabora (67) recorded venous pressures as high as 320 mm. of water in decompensation and stated that values of 200 to 250 are not infrequent. Frank and Reh (89) found high venous pressures in two cases of decompensation, falling to normal as compensation was regained. Clark (100) studied fourteen cases of cardiac decompensation in detail and concluded that venous pressure observations were of great value in estimating the degree of decompensation and in determining prognosis and the results of therapy. He also showed that the urine output during decompensation varied approximately inversely as the venous pressure. He proposed 200 mm. of water as the critical level in decompensation. Above this level a rising venous pressure has an unfavorable, a falling venous pressure a favorable prognosis. Fuchs (62), using the direct method, observed venous pressures of from 70 to 215 mm. in eight cases of decompensation and in one severe case dying shortly after a rise of 325 mm. was present. While these figures are usually high as compared with the normals established by this investigator (76 mm. of water), he was unable to associate the height of the venous pressure with the extent of decompensation, and believed the latter is better judged by the general clinical picture than by the height of the venous pressure. Villaret, St. Girons and Grellity-Bosviel (80), (101), (17) using the same method, found invariably an abnormally high venous pressure in decompensation and state that a rising pressure may be a very early sign of impending heart failure. Venous pressures of 300 to 400 mm. of water were noted. Castellotti (82), with the same method reported four decompensated heart cases with a venous pressure reading of 137 mm. and variations between 130 and 160 mm. of water, and stated that marked venous hypertension occurred exclusively in this condition. Ratky and Klein (103), working also with the direct method of measuring venous pressure, found a high pressure in cardiac decompensation but also in certain other conditions. They compared the venous pressure, clinical findings and capillary flow by the Weiss (104) method for estimating the functional condition of the circulation. Con-

clusions from these different methods agreed in severe decompensation, but in milder decompensation and in other conditions marked disagreement was found. They stated that in decompensation the venous pressure usually but not always is elevated in proportion to the clinical severity of the heart failure. With the same method of measurement, Kroetz (63) concluded that while venous pressure was nearly always high in severe decompensation, the severity of the condition was not paralleled by the rise, that in moderate decompensation normal venous pressure readings were not infrequent, and that abnormally high venous pressures were present in other conditions without evidence of heart failure. This observer laid great stress on the influence of intrathoracic pressure as affecting peripheral venous pressure, and believed that when the former is altered by deformity of the thorax (empyema, pneumothorax, scoliosis), or by disease of the lungs (emphysema, pleuritis, pleural adhesions), that the venous pressure as read in the peripheral vein may be no index of the "effective venous pressure" at the heart. He divided his cases of mild cardiac decompensation into two groups, one in which the thorax and lungs are normal and which show a moderate rise of venous pressure, and the other in which intrathoracic conditions determine a peripheral venous pressure below normal, although the "effective venous pressure" (right auricular pressure) was probably high. In two cases in which artificial pneumothorax was produced, he measured intrathoracic pressure and found alteration in venous pressure with the altered intrathoracic pressure, the effective venous pressure being maintained, however, at a practically constant figure. Kroetz also sought to determine whether or not changes in carbon dioxide content of the blood occurring in cardiac and pulmonary conditions, affected venous pressure. He estimated arterial and venous carbon dioxide tensions by the Plesche and Haldane methods, but was unable to associate the changes observed with any significant changes in venous pressure. He concludes that in certain cases variations in the clinically unmeasurable intrathoracic pressure nullifies the diagnostic and prognostic value of the clinically measurable venous pressure. Other extracardiac factors that may modify peripheral venous pressures, according to this investigator, are strong muscle development and adipose tissues, producing an effect by direct pressure on the veins. Eyster and Middleton (95) using the indirect method of measurement, found an invariable rise of venous pressure in cardiac decompensation, and confirmed the findings of Hooker and Eyster (86) and of Clark (100), as to its relation to the extent of decompensation and fall during restor-

ation. They also confirmed Clark's (100) findings as to the relation of venous pressure and urine output, and established an approximate concordance between the rise of venous pressure in decompensation and cardiac dilatation as determined by teleroentgenographic studies.

Venous pressure changes as a result of exercise have also been shown to be different in decompensation than in the normal subject. Schott (77) found an abnormal rise of venous pressure in eight decompensated cases on elevation of the leg. The venous pressure in these cases varied between 66 and 283 mm. of water. White, Barker and Allen (102) have recently studied the venous pressure reaction to graded dynamic exercise, and found that in decompensation the rise is higher and the pressure fails to fall to normal during the exercise or immediately on its cessation,—the reaction typical for normal subjects.

That the peripheral venous stasis and high venous pressure present in cardiac decompensation may account in part for the cyanosis in this condition, independently of changes in oxygen content of the blood, is probable from the work of Goldschmidt and Light (105). These investigators found that when the hand of a normal subject is held well below heart level, the engorgement of the capillaries and venules causes marked cyanosis without any increase in the oxygen unsaturation of the blood in these vessels. According to Danzer and Hooker (106), it is the venous plexuses which normally cause the color of the skin, and blanching produced by pressure results from the emptying of these vessels. This conclusion has been confirmed by White (15).

Effect of therapy in decompensation. A study of the influence of drugs used in cardiac therapy upon venous pressure has been made in the experimental animal by Capps and Matthews (107). In the normal animal, digitalis and caffein caused a moderate and gradual rise of arterial with no effect upon venous pressure. Strychnine and morphine had no effect in therapeutic doses. The nitrites caused a fall of both arterial and venous pressures. Bedford and Wright (64) noted a slight rise in venous pressure (20 to 30 mm. of water) in man on inhaling amyl nitrite, and Payan and Geroud (90) reported a brief rise followed by a fall from amyl nitrite and erythrol tetranitrate. The influence of combined therapy in cardiac decompensation (rest, digitalization, diet, fluid control) has been noted by numerous workers. Villaret, St. Girons and Grellity-Bosviel (17) stated that digitalization may produce a fall of from 150 to 200 mm. of water, and that a less marked but more rapid fall followed the injection of oubaine intravenously. Observations have also been made on the effect of venesection in decompensation. In a

case reported by Hooker and Eyster (86), the venous pressure fell immediately from 260 to 210 mm. of water and subsequently to normal, following the withdrawal of 200 cc. of blood. Fuchs (62) reported a fall in venous pressure from 185 to 145 mm. of water in a pneumonia, and from 123 to 85 mm. of water in an apoplexy, following venesection. In a case of sublimate poisoning with a normal venous pressure the pressure fell only 20 mm. of water following venesection. Villaret, St. Girons and Grellity-Bosviel (17) stated that venesection may cause a fall of 150 to 200 mm. of water in decompensated cases. Kroetz (63) reported one case of decompensation in arterial hypertension, in which venous pressure fell from 110 to 20 mm. of water as a result of venesection, but returned the next day to 100 mm. Eyster and Middleton (95) called attention to the fact, that while in the normal human withdrawal of moderate amounts of blood produces only transient effects on venous pressure (42), in decompensation the venous pressure may show a marked and permanent fall. Falls of from 220 to 50 mm. and from 180 to 60 mm. of water were reported. They gave the indication for venesection as a high and rising venous pressure. The writer feels that this is one of the greatest values of the routine and frequent determinations of venous pressure in cardiac decompensation, and because of this and the fact that, so far as he knows, detailed observations have not been reported, he ventures to record briefly a few recent and hitherto unpublished observations made in the medical service at the University Hospital. In seven cases of severe decompensation, four of primary valvulitis and three of hypertension and myocarditis, venous pressure on admission (at bed rest) varied between 150 and 280 mm. with an average of 210 mm. of water. Immediately following the withdrawal of from 100 to 500 cc. of blood, the pressures fell to an average of 130 mm. The percentage fall in the different cases varied from 20 to 50, with an average of 37 per cent. Venous pressure, shortly after the initial fall, rose in five cases, three of whom died. It continued to fall in two cases, one of whom subsequently died with a terminal rise of venous pressure a few days before death. In the three cases in which compensation was restored, there was a rapid and permanent increase in urine output and a rise of vital capacity following the venesection. In the one case showing a fall of venous pressure lasting until a few days before death, there was a marked but temporary improvement. The other three cases showed no increase in urine output or in vital capacity. Venesection was done in four cases only after a prolonged stay in the hospital, and in whom no improvement had followed rest, digitalization and diet.

Three of these died. Venesection was done shortly after admission in three cases, one of whom died.

Paroxysmal tachycardia and fibrillation. Hooker and Eyster (86) reported a case of paroxysmal tachycardia in which the venous pressure rose from the normal of 105 to 210 mm. of water during an attack. Arterial pressures were changed from 135/85 to 120/110 mm. of mercury. The pulse rate during the attack was 220. In a similar case reported by Kroetz (63), the venous pressure was 160 mm. during the attack, falling to 55 mm. of water on its cessation. The heart rate during the attack was 220 as compared with a normal of 70. There were signs of venous stasis in this patient during the fast heart rate (cyanosis, dyspnea, liver engorgement, albuminuria). Eyster and Middleton (95) found on the other hand, a rise of only 10 mm. of water (from 80 to 90) in an attack of paroxysmal fibrillation unattended with signs of stasis. The ventricular rate increased from 84 to 108, and the size of the x-ray silhouette of the heart diminished during the attack. It would seem, therefore, that venous pressures may or may not rise in paroxysmal tachycardia and fibrillation, depending upon whether or not cardiac incompetence supervenes.

Compensated heart disease. According to most observers, venous pressure in compensated heart disease is within normal limits. Frey (83) stated that a slight rise may be the first objective sign of impending failure. Schott (77) in his study of the influence of static exercise (elevation of leg), found a higher rise of venous pressure in compensated cardiacs than in normals. White, Barker and Allen (102), on the other hand, were unable to demonstrate any difference between the venous pressure response to dynamic exercise in compensated cardiac patients and normal subjects. Fuchs (62), using the direct method of venous pressure measurement, recorded normal venous pressures in five cases of compensated heart disease. Kroetz (63), with the same method, found an average venous pressure of 72 mm. with variations between 72 and 135 mm. of water in eleven similar cases. These included two cases of auricular fibrillation of long standing. Villaret, St. Girons and Grellity-Bosviel (80) found normal venous pressures in compensated cases except in aortic regurgitation, where the pressure was usually somewhat elevated, and concluded that compensation in this condition is not always as perfect as it appears to be. In five cases reported, four showed venous pressures between 190 and 240 mm. of water. Frank and Reh (89) specifically stated that the venous pressure is normal in compensated aortic disease, and Le Conte and Yacoel (78)

failed to substantiate the findings of Villaret, St. Girons and Grellity-Bosviel. Castellotti (82) reported five compensated cardiacs with an average venous pressure of 72 mm., a figure at the upper limit of the normal established by this worker. The figures varied in these cases between 60 and 90 mm. One was a case of mitral and aortic regurgitation with a venous pressure of 85 mm. Eyster and Middleton (95) found normal venous pressures in compensated cardiacs, but stated that in cases showing clinical evidence of poor compensation, the venous pressure was usually in the upper limits of the normal range with an average above the usual normal subject.

Arterial hypertension. Numerous experiments have been quoted in the preceding pages, which indicate that under many conditions at least, changes in arterial pressure have little influence upon venous pressure. Some observers however have found in man a relation between the two, both in the normal and in arterial hypertension. Sewall (61) recognized two groups of arterial hypertension, in one of which venous pressure was low, in the other high. The former included cases of "arterial spasm" without palpable arteriosclerosis, and cases of aortic sclerosis, the latter conditions characterized by "plethora," especially when associated with retinal hemorrhage. Brown (88) recorded normal venous pressure in two cases of "essential" hypertension. Villaret, St. Girons and Grellity-Bosviel (80) found that in normal subjects, and in compensated heart conditions, venous pressure was proportional to systolic arterial pressure, the relation being approximately as 1:13. They again recognized two groups in arterial hypertension, but with apparently opposite findings to those of Sewall. In cases of hypertension where there was little evidence of arteriosclerosis, venous pressure was high. In another group, with well developed arteriosclerosis, venous pressure was below the normal range. They explained their findings by the transmission of the "vis et tergo" of the high arterial pressure through the peripheral vessels, when these were approximately normal, while if they were narrowed by disease, this factor had less effect upon venous pressure than in normal cases. Le Conte and Yocoel (79), using the same method of determining venous pressure, were unable to confirm this finding, and stated that venous pressure rises in hypertension only as cardiac decompensation appears. Fuchs (62) found an average venous pressure of 56 mm. of water in six cases of arteriosclerotic hypertension. It was within normal limits in all. Ratky and Klein (103) presented a group of eight hypertensions in which they stated that there were no objective or subjective signs of

heart failure. Venous pressure, measured by the direct method, gave values from 90 to 210 mm. of water, with an average of 150 mm. In two subjects, considering their age, an arterial hypertension could hardly be said to exist, and in one of these a venous pressure of 180 mm. of water was recorded. There was no relation between the extent of the arterial and venous hypertension in these cases. They concluded that in arterial hypertension, where usually an obviously high degree of irritability of the vasoconstrictor mechanism exists, it is not improbable that the venomotor system is also hyper-irritable and that over-activity of this mechanism may explain the high venous pressure. Castellotti (82) divided the arterial hypertension cases that he has observed into three groups. The first included those in which the arterial changes were advanced, the arterial pressure high and the heart well compensated. In these venous pressure was below normal (15 to 30 mm. of water). The second group was that of moderate arterial hypertension with good cardiac compensation and normal venous pressure. The third group comprised arterial hypertension with poor cardiac compensation, and with venous pressure in the upper range of normal or above. Helouin (108) observed arterial and venous hypertension occurring together in fifty cases, but stated that a high venous pressure in hypertension is an indication of cardiac insufficiency, while a low venous pressure in the same condition is indicative of good compensation. Tatterova and Serf (92) found the venous pressure normal in arterial hypertension.

Lobar pneumonia. Schott (77) found a normal venous pressure response to static exercise (holding leg elevated) in pneumonia when the heart was not involved. Fuchs (62) measured the venous pressure in nine cases of pneumonia. Eight of these had normal venous pressures, two dying within thirty hours after the pressures were determined. The other case had at first a venous pressure of 73 mm. of water, rising to 173 mm. and falling to 135 mm. as a result of venesection. Death occurred the following day. Levy (109) found evidence of cardiac dilatation in 60 per cent of twenty-one cases of lobar pneumonia without cardiac decompensation. Venous pressure was not measured as a routine, but was found normal in the cases where it was determined. Eyster and Middleton (95) reported frequent venous pressure determinations in eight cases of lobar pneumonia. In six, the readings were normal throughout the course of the disease, and there was no evidence at any time of cardiac incompetence. In two cases, periods of cardiac failure supervened, and in each it was associated with an abnormally

high venous pressure. In one of these cases, parallel observations of heart size (teleroentgenographic) and capillary flow were made. During two periods of cardiac failure in this case, there was no demonstrable change in heart size, but a definite capillary stasis and high venous pressure developed, disappearing as compensation was restored. These observers concluded that an abnormally high venous pressure in pneumonia is indicative of cardiac decompensation.

Pulmonary conditions other than pneumonia. Gaertner (91) found a high venous pressure in advanced tuberculosis and in pleuritic exudates, and recorded one figure of 180 mm. of water in a massive emphysema. He failed to note whether any of these cases showed clinical signs of cardiac incompetence. Fuchs (62) found a moderate rise of venous pressure (average pressure of 115 mm. of water) in fourteen cases of tuberculosis where arterial hypotension was present. In other cases the venous pressure was normal. Castellotti (82) found a normal venous pressure in pulmonary tuberculosis of slight or moderate grade, and a high venous pressure in advanced tuberculosis with arterial hypotension and evidence of cardiac incompetence. He stated that the cardiac effects in pulmonary tuberculosis arise from 1, pulmonary stasis due to mechanical factors, and 2, direct or toxic involvement of the heart. Villaret, St. Girons and Grellity-Bosviel (17) found a transient rise of venous pressure in emphysema during periods when acute pulmonary edema occurred, and stated that this is not due to cardiac failure, but to a rise of tension in the pulmonary circuit. Venous pressures were normal in chronic emphysema. Markovitch (110) found venous pressure occasionally elevated in the latter affection, and when elevated it was accompanied by x-ray evidence of dilatation of right heart, but without signs of failure. Kroetz (63), as has been previously stated, laid great stress upon the influence of intrathoracic conditions on peripheral venous pressure. In general he found low venous pressure in emphysema and normal venous pressures in asthma and pleural exudates. This observer believed that in deformities of the thorax and in emphysema the venous pressures may be so much reduced by the intrathoracic factor, that they may still be within the normal range, notwithstanding the presence of cardiac decompensation. Ratky and Klein (103) reported one case of pleuritis in which there was a high venous pressure, ascribed by them to a reduction of the aspirating influence of normal respiration. Villaret and Martiny (111) studied the venous pressure in different types of pulmonary tuberculosis and found a rise in the miliary and fibroid types with normal pressures in the other

forms. High venous pressures in mediastinal growths and in struma, probably due to direct pressure on the veins, have been noted by Burger (68), Kroetz (63) and Eyster and Middleton (95). It should finally be noted that Plumier (13) found in the dog that injection of fluid into the pleural space caused no rise of venous pressure until the period immediately preceding death, at a time when asphyxia had resulted from compression of the lungs by the fluid.

Peripheral vasodilatation. The absence of a change of venous pressure in local vasomotor changes induced in the hands by heat and cold (61), (14), (15) and in the extreme vasoconstrictions of the "Raynaud phenomenon" (16) has already been noted. Kroetz (63), however, found a mean venous pressure of 90 mm. of water in twenty-three cases classified as vasomotor instability. Over half of these cases were above the upper normal limit accepted by this investigator (80 mm. of water). Eyster and Middleton (95) failed to find any noteworthy change in venous pressure in acute febrile conditions with relaxed peripheral tonus. In a series of cases of acute rheumatic fever, in which this condition is developed to a pronounced degree, capillary pressure as measured by the method developed by Danzer and Hooker (106) was definitely lowered, while venous pressure was normal.

Local venous obstruction. Local obstruction to venous return will cause a rise of pressure in the affected vessel. Frank (112) found that on obstructing venous return from the arm by an inflated cuff, venous pressure peripheral to the obstruction rose from 50 to 120 mm. of mercury (670 to 1620 mm. of water). Pressures this high have never been recorded clinically. Kroetz (63) referred occasional high venous pressures in normal individuals to excessive muscular development compressing the peripheral veins, and the high venous pressure found by the same author and by Ratky and Klein (103) in obesity, is apparently ascribed to the same factor. Hooker and Eyster (86) mentioned phlebosclerosis as the occasional cause of high venous pressure readings, but this appears to be rather rare (95). Local obstruction by mediastinal growth has been mentioned. In one case of Hodgkin's disease with extensive mediastinal involvement, Eyster and Middleton (95) observed a gradual rise of venous pressure in the veins of the hands from 80 mm. of water early in the disease, to 200 mm. as the condition progressed. Venous pressures in the veins of the lower extremity were normal throughout. Autopsy revealed partial occlusion of the superior vena cava. Runge (18) found a rise in the venous pressure in the leg veins in gravid women, even in the early months, falling to below normal

in the puerperium. Pressures in the arm veins were little affected. Villaret, St. Girons and Salasc (113) found a rise of venous pressure in varicose veins when the condition was the result of obstruction. When not due to obstruction venous pressures were lower than normal in the prone position, but rose to enormous values in the erect (990 mm. of water), ascribed to loss of tonus in the vessels. La Conte and Yocael (79) found a venous pressure of 190 mm. of water in the varicose veins of the lower extremity in one case in the prone position. Middleton (114) reported venous pressure readings approximately twice as high in the veins of the left hand as in those of the right in a case of aortic aneurism, presumably due to obstruction to venous return. Ayerza (115) records the highest venous pressure in the clinical literature, 490 mm. of water in a case of polyserositis involving the pleura, pericardium and peritoneum, presumably the result of stasis from pressure.

Miscellaneous conditions involving the cardiovascular system. In acute or sub-acute endocarditis, the venous pressure is normal unless cardiac failure supervenes (95), (115). In arterio-venous aneurism, the general venous pressure is increased, but may be higher in veins draining the region where the anastomosis occurs, than in those from other regions (116), (115). Ayerza (115) reported one case of dry pericarditis with an approximately normal venous pressure. So far as the writer knows, no cases of pericarditis with effusion, and without signs of cardiac failure, have been reported. Pernicious anemia, although associated with marked changes in cardiac output and blood flow (117) exhibits a normal venous pressure (89), (95).

Miscellaneous disease. Gaertner (91) noted a venous pressure of 300 mm. of water (the highest he observed) in a 30-year-old woman with cachexia, high fever and apical infiltration. The cause of the rise was not clear. v. Basch (84) and Moritz and Tabora (67) recorded various diseases other than cardiovascular with normal venous pressure readings. Ratky and Klein (103) found a high venous pressure in three cases of carcinomatosis. Ayerza (115) on the other hand, found a normal venous pressure in a case of pulmonary neoplasm. Eyster and Middleton (95) failed to find any noteworthy elevation of venous pressure in diseases not involving directly or indirectly the cardiovascular system. Villaret, St. Girons and Grellity-Bosviel (118) have described a new clinical syndrome, showing as its main features cyanosis, venous hypertension and evidence of ovarian insufficiency, and occurring principally in young women.

DISCUSSION

A review of our present knowledge concerning the physiological control of venous pressure emphasizes the importance of two factors which have become fully evident only within recent years, a variable vascular bed accommodating varying amounts of blood without necessarily accompanying pressure changes, and a cardiac mechanism regulated as to the degree of its activity, at least in large part, by the venous return. Both of those mechanisms tend to stabilize the venous pressure. The former may be regarded as a buffer protecting the heart against extreme variations in venous load, the latter as an adaptable mechanism by which the extent of arteriole and capillary circulation is largely determined by the return flow from the peripheral vessels to the the heart. These two mechanisms together assure that under normal conditions, heart load will not exceed the range of physiological response of the cardiac muscle, and that the heart will pump into the arterial side of the circulation the blood that comes to it from the veins without more than a temporary rise of venous pressure. This mechanism appears to function well even under severe physical activity (cf. White (98)). When we are concerned with a heart muscle in which the range of physiological response to increasing load has been narrowed as a result of disease, the response may fail under a high venous load, and a persistent venous engorgement result, even though the venous return is reduced to a low limit. This constitutes the condition which we designate as cardiac failure or decompensation and one cannot conceive of this developing, except after severe blood depletion or in vascular paralysis (vascular shock), without an abnormal and persistently high venous pressure. Ventricular incompetence supervenes when the initial load on the ventricle, represented by the venous pressure, exceeds the physiological limit of the muscle to respond to an increased load by increased work. Cardiac decompensation is not ordinarily accompanied by an increase in the general blood volume (119), but the attending vasoconstriction, by which a normal or increased arterial pressure is maintained in the presence of a diminished cardiac output, and the failure of the heart to move the usual amount of blood from the veins into the arteries, results in a stagnation of the blood in the veins and a proportionate increase of blood volume in this part of the circulation.

While the heart muscle under conditions of decompensation actually requires more oxygen than normally per unit of work performed, its own supply of oxygen is greatly reduced. The slowing of the pulmonary

circulation leads to deficient blood aeration, while the heart muscle requires more oxygen in proportion to the mechanical work it performs than under normal conditions of initial tension (120) and oxygen supply (121). The coronary blood flow is also reduced, as a result of the dilatation of the muscle (122) and the rapid and weak contractions (123), (124). A further reduction in cardiac efficiency, probably largely the result of anoxemia, thus tends to occur, and this leads to still further venous stagnation and increase of the venous load. The "vicious circle" of cardiac decompensation becomes established. The therapeutic indication for the reduction of muscular and other activities to as near basal conditions as possible, is obvious from the standpoint of the oxygen requirements of the tissues, and in lowering the demand for peripheral blood flow and consequently the venous return to the heart. The value of venesection in reducing the proportionately high venous blood volume and the abnormal initial load on the right ventricle is equally clear. The improvement of the heart under digitalis may be due in large part to the relief of anoxemia by the slower rate produced. Loevenhart, Malone and Martin (125) have shown that the increased heart rate as a result of removal of vagal inhibition from a partially asphyxiated heart, has a very deleterious effect and leads quickly to complete heart failure. Improved oxygen utilization by the cardiac muscle, increased coronary flow resulting from slower, stronger contractions, and the lowered venous pressure offering less hindrance to the return flow from the coronary sinus are important factors in reducing the anoxemia of the heart muscle as compensation begins to be restored. Ventricular competence is regained when, by improvement in the heart muscle and by reduction of the venous engorgement by rest, venesection or other measures, the muscle begins to respond to the increased load by increased work. As the muscle becomes more efficient in its response, the venous load diminishes, and cardiac output and venous pressure return to within their normal ranges. With our present understanding of the situation it is impossible to conceive of a high and sustained venous pressure in the presence of a competent cardiac muscle. Reported clinical observations of such a condition with the absence of other evidences of cardiac incompetence, are few and scattered, and may be explained most probably on faulty venous pressure determinations or the presence of an unrecognized cardiac incompetence.

A consideration of the fundamental physiological basis of venous pressure, the clinical reports in the literature and his own experience, leads the writer to express his conclusion, that excluding local causes

of venous obstruction, a high and sustained venous pressure invariably means cardiac failure, independent of the conditions leading to the failure or the associated disease.

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THE PALEOCHEMISTRY OF THE BODY FLUIDS AND TISSUES

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I. Introduction—General. In the development of the ovum of any one of the higher Metazoa there occurs a succession of changes and transformations in structure, typical of the embryonic stage of all such forms, in which one can find indications of its remote ancestral history. The order in which the organs develop constitutes a succinct repetition of the course of the evolution of the organism from a very much simpler one through many millions of years. Each organ is an acquirement of its own time in that long descent and as such it has been either further developed, or even maintained, when, as in the case of the notochord, its function has lapsed. On its morphological side, accordingly, the organism has fixed endowments of an origin as remote as the Cambrian of geological time.

Even in the cell, the unit of living matter, endowments undoubtedly of much more ancient origin are to be found. The cell nucleus was an acquirement of a date much anterior in time to the appearance, most probably in the incredibly long period known as the pre-Cambrian, of at first very simply organized Metazoan and Metaphytan forms, by non-nucleated cells when these were not as yet differentiated as animal or vegetable. This explains why in the phenomena of mitosis there is so much that is common to animal and vegetable cells and we can find the origin of it in the differentiation and specialization of one of the several simple more or less amitotic modes of nuclear division which must have then obtained, exemplifications of some of which are still to be found in the atypical nuclear divisions in a number of the Protists, notably *Euplotes*, *Ceratium*, *Euglena* and *Calcituba*. The cell, therefore, on its morphological side, has endowments transmitted from a past almost as remote as the origin of life on earth.

The cell, animal and vegetable, and the Metazoa, so far as structure is concerned, having thus endowments of primeval origin, it may be asked whether there are not also in unicellular animal and Metazoan forms, endowments, on the chemical side, of equally ancient origin.

In the living complex of every cell the basic elements, proteins, composed as they are each of a variable number of amino acids and in variable proportions of these, cannot be predicated as having a uniform composition even absolutely in similar cells in the same organism, for thus mutation would never obtain in a species. This mutability of protein may explain the origin of the cell nucleus as a sanctuary to maintain, in a measure, the uniformity in composition of the chromatin which determines the transmission from cell to cell, from organism to organism, of the characters, inherited from a far past, which make for stability in the species. Even chromatin, which is always an iron-holding nucleoprotein basically, is not uniform in composition from class to class, or genus to genus, or even in closely related species, as, otherwise, the transmission by it, in germ cells, of specific characters would be inexplicable.

Beyond, then, the living complex, which is always constituted of proteins, but not uniform in composition in the wide range of cellular life, there are no organic compounds whose constancy of occurrence in living matter can be regarded as an endowment from the far past.

There remain the inorganic constituents. Of the nine or more inorganic elements which have been found to be constantly associated with living matter, four—sodium, potassium, calcium and magnesium —occur as chlorides, sulphates, phosphates and carbonates in the living cell and in its media and in porportions the constancy of which, within certain limits, indicates that they are of more than casual origin and that they may be of a paleogenetic character. The relative proportions of these elements are, however, not the same in the cell as in its media. In the cell the potassium is much the more abundant; thereafter, in order, come sodium, magnesium and calcium, while in the media (blood plasma and lymph) the sodium far exceeds in amount the potassium, and the calcium is more abundant than the magnesium. In the other media, ocean and river water, the proportions are different yet the sodium is always in excess of the potassium, and in sea water the magnesium greatly exceeds the calcium and potassium, but apart from the magnesium the relative proportions are approximately those obtaining in blood plasma and lymph.

At once it is suggested that as the cell is older than its media as at present constituted, the relative proportions of the inorganic elements which obtain in it are of more ancient origin than the relative proportions of the same elements which prevail in the media, blood plasma and lymph or in the ocean and river water of today.

One might then postulate that on the inorganic side of living matter there are two endowments from the remote past. One of these may hark back to the period in the history of the earth when all life was unicellular and when even a cell nucleus had not as yet been evolved. The other may be considered as dating from the time when Metazoan organisms, in which the internal medium was the then ocean water, acquired a closed circulatory system and developed organs which regulated and kept constant, within certain limits, the inorganic composition of the internal medium whatever changes in composition the external medium, the ocean, underwent in subsequent time. This latter endowment may be held to be exemplified in the Vertebrate.

It is the purpose of this contribution to discuss the character of the evidence available at present which tells in favour of or against the conception that there are such endowments, which may be called paleochemical, of animal organisms, endowments which would predicate that all the serried ages of the earth's history do not sleep in stone alone.

II. Earlier literature on the subject. The earliest references to possible paleochemical endowments of living matter were those made by Bunge and Quinton.

Bunge, in 1889, discussing the significance of the occurrence, in the tissues of Vertebrates, of sodium chloride in quantities exceeding those of potassium and other elements, and the constant need of this salt in our diet, advanced the view that it can be interpreted only through the application of the theory of Evolution (Descendenzlehre). All life began in the primeval ocean and all the primal Vertebrates were sea forms. In the ocean from the beginning sodium chloride greatly exceeded the other salts therein and, in consequence, during the long ages in which Invertebrates and primal Vertebrates had a marine habitat, their tissues accommodated themselves to the saline medium, and ultimately in land Vertebrates, ages later, maintained the relationship to salt so developed.

The predominance of sodium in ocean water was the result of the interaction of the negative elements for the possession of the bases in primal conditions on the earth's surface. In this struggle (Kampf) carbonic acid manifests a great affinity for sodium and silicic acid for potassium. In the weathering of the silicate rocks the sodium set free in the decomposition goes as carbonate into solution and finally into the sea where it interacts with the chlorides of the alkaline earths to form the sodium chloride of the ocean and the carbonates of calcium

and magnesium which on deposition form the limestones, chalks and dolomites in the rock strata. Potassium, on the other hand, with its special affinities for silicic acid along with certain other basic elements, notably aluminum, forms insoluble double salts as silicates which constitute the siliceous rocks. This explains not only the excess of sodium in sea water but also its low potassium content, the low sodium chloride content, apart from the salt deposits derived from evaporated sea water, of the land surface of the globe and the high potassium and low sodium contents of land plants. Land plants are thus in contrast with land animals, excepting the Insecta, which Bunge found to contain relatively no more sodium than the plants from which they derive their nourishment. The high sodium chloride content of land animals, and especially land Vertebrates, is still maintained, even in an environment wanting, or almost so, in this salt, ages after their ancestral forms abandoned the sea.

Summing up, Bunge says: "Thatsächlich hat doch jeder von uns in seiner individuellen Entwickelung ein Stadium durchgemacht, in welchem er noch die Chorde dorsalis und die Kiemenspalten der Meerbewohnenden Vorfahren besass. Warum sollte nicht auch der hohe Kochsalzgehalt unserer Gewebe ein Erbstück aus jener Zeit sein?"

In explaining thus the origin of the sodium chloride endowment of Vertebrates Bunge does not distinguish between the tissue elements, the cells, and the fluid, the internal medium, which constantly bathes them. He notes that the Vertebrate embryo is richer relatively in sodium chloride than the new-born animal and that after birth it becomes even poorer, relatively, in sodium and chlorine as development proceeds. This decrease in sodium chloride he seems to indicate as due to replacement of some of the tissues of more ancient origin by tissues developed to meet the requirements of the adult in an altered or very different environment, as for example, cartilage by bone. Cartilage is of remote origin, as shown by the fact that in Selachians it persists and constitutes the only skeletal tissue they have, and cartilage wherever it occurs is rich in sodium. He does not refer to the inorganic contents of tissue elements of equally ancient origin, nerve cells, liver cells and muscle fibres, and it might be inferred that they are also rich in sodium but for his observations on the potassium in animal and vegetable cells. The sodium chloride content of the environment dominates the sodium chloride content of the organism. It is otherwise, he points out, with the potassium, which is "ein integrirender unentbehrlicher Bestandtheil" of every plant and animal cell and every cell

has the capacity to pick up and assimilate this element in the necessary quantities from environments which may contain traces only of potassium. Why the living complex which constitutes the essential of the animal and vegetable cell should be so selective of potassium he does not explain beyond what is meant by the adjectives, "integrirender" and "unentbehrlicher."

Quinton, in 1897, advanced the view that in the great majority of multicellular animal organisms the internal medium, the circulatory fluid, or hemolymph, is, as regards its organic composition, but sea water. This view he elaborated in more detail in his later publications on the subject. With Bunge he postulated the origin in the sea of all animal life whether unicellular or Metazoan, and he finds, by census as it were, that the vast majority of animal forms are still marine. In some of these, for example the Porifera, the Hydrozoa and the Scyphozoa, in which the circulation is open to the exterior the internal medium is but sea water which bathes all the cells of these forms. In other marine Invertebrates the circulation is closed off from the exterior but the external surface of these is permeable readily to ocean water and its salts, so accordingly the internal medium is still but ocean water. In all Invertebrates of fresh water habitat, except sponges and Hydrozoa and the mussel, *Anodonta cygnea*, which are degenerate forms, the internal medium (*milieu vital*) is, in its saline composition, constant and specific and chemical analysis shows it to be marine in character, as it is also in non-aquatic Invertebrates. Even in the highest representatives, i.e., the Vertebrates, of the zoölogical series, the most remote (mammals and birds) in time from the life in the sea, the vital medium of the cells is identical with the marine medium.

This thesis Quinton endeavours to support by the result of experiments on Vertebrates in the circulation of which he injected large quantities of sea water rendered isotonic with blood plasma by dilution with distilled water, and noted the effects. The animals did not exhibit any abnormality, or if a peculiarity a negligible one, and only temporary in character. Into the circulation of dogs "bled white," that is, to death if not treated further, sea water rendered isotonic was injected in quantities large enough to replace the blood lost. These animals were next day very active and at the end of several days normal and the hemoglobin of their blood reconstituted. The fresh white blood corpuscles of representatives of the different classes of Vertebrates were submerged in blood plasma diluted with 1-25-50-100 units of sea water and in every case they remained normal.

Analysis of the salts of the blood plasma, Quinton holds, indicates that they are the same as those which obtain in sea water and the elements in both appear in the same order of importance: Chlorine, sodium, potassium, calcium, magnesium, sulphur, silicon, carbon, phosphorus, fluorine, iron, nitrogen, etc. Even in the elements which obtain in minute traces in sea water and in the vital medium: iodine, bromine, manganese, lead, etc., there is a parallelism.

This indicates how uncritical he is in the examination of his data in his aim to demonstrate that the internal medium is but sea water. The elements do not appear in the same order of importance as stated. In sea water they rank thus: chlorine, sodium, magnesium, potassium, sulphur, calcium, etc., whereas in the blood plasma they rank: chlorine sodium, potassium, calcium, sulphur, magnesium, etc., In sea water the sodium is to the magnesium in amount as 100:12, whereas in the blood plasma of the higher vertebrates the ratio is as 100:0.7, which reveals a wide discrepancy. As regards the sulphur, which occurs almost wholly in sea water as sulphates, it is in amount in proportion to the sodium as 8.4:100, whereas in mammalian blood plasma if all the sulphur therein is reckoned as present in the form of sulphate, the proportion is 1.4:100.

The concentration in salinity of the internal medium varies in different Vertebrates and Invertebrates. In marine Invertebrates it is approximately or actually that of sea water. In fresh water Invertebrates, e.g., *Astacus*, it is less than 40 per cent of that of sea water, while in the higher Vertebrates (mammals) it is less than one-fourth of that of sea water. Nevertheless, Quinton holds, in all these the internal medium is sea water, chemically considered, and the concentration, which determines osmotic pressure, is purely physical, just as the air at the summit of a high mountain is still air although its pressure at that elevation is less than that of the air at low levels. The variations in concentration arose through changes in habitat or mode of life and the difference in concentration from that in sea water has thus become marked in mammals, in which, as they, the most highly developed of all the Vertebrates, are the most remote in time from the ancestral marine stock, the concentration in the plasma through the long ages has progressively undergone a decrease. What the original concentration was must have been approximately that of the ocean of to-day for Quinton holds that the composition of modern sea water is very near ("très voisine") that of pre-Cambrian seas in which animal life first made its appearance. In support of this view he refers to analyses of the salt

deposits from the seas of the Permian and Triassic periods as indicating such a similarity in composition of the pre-Permian and modern seas. It may, however, be pointed out that though there may be such a similarity as Quinton predicated, the physical conditions under which salt deposits may be formed, as viewed by the physical chemist, are not necessarily of such a character as to make these deposits document the composition of the ocean of the time of their formation. Further, it may be held that if the composition of the ocean of the Permian period were thus ascertainable it would not demonstrate, necessarily, what was the composition of the ocean of the pre-Cambrian period. Ocean water through all the ages has been changing, not only as regards its saline concentration but also as to the relative proportions of its salts, and the sea water of the pre-Cambrian period would, therefore, in its saline composition be appreciably different from the sea water even of an age as early as the Permian. Even in the incredibly long pre-Cambrian period the saline composition and concentration of the ocean must have been very different at the beginning from what they were at the end. These considerations must be regarded as fundamental in any attempt to explain the origin of the inorganic composition of the circulatory medium in Vertebrates and Invertebrates of today.

III. The author's previous observations. In 1903, in discussing the bearings of the results of an investigation of the inorganic composition of the Medusae, *Aurelia flavidula* and *Cyanea arctica*, which he had then completed, the author of the present contribution, quite unaware of the speculations and views of Bunge and Quinton, as abstracted above, advanced the view that the blood plasma of Vertebrates and Invertebrates with a closed circulatory system is, in its inorganic salts, but a reproduction of the sea water of the remote geological period in which the prototypic representatives of such animal forms first made their appearance. It was pointed out that in many Invertebrates with a vascular system still communicating with the exterior the circulatory fluid is sea water and that this was very probably the case also with ancient oceanic forms. The tissues in the latter, it was represented, had through a long period of time become so accommodated to the composition of the then sea water that when the circulatory system acquired the closed condition, the composition of the sea water of that time was, with slight modifications, reproduced in the vascular fluid and thus transmitted to the descendant forms living in similar or different habitats.

It was shown that even between the inorganic composition of the

blood serum of mammals, as the analyses then hitherto made indicated, and that of the ocean of to-day there is a striking resemblance. This does not hold for concentration, for the salinity of the ocean is more than three times that of mammlian blood serum, but it does in the proportions in the latter of potassium and calcium relative to sodium, the most abundant basic element therein, as indicated thus:

	Na	K	Ca	Mg
Ocean	100	3.66	3.84	11.99
Mammalian blood serum	100	6.69	2.58	0.8

The resemblances are very close except in the case of the magnesium, but this exception and the minor differences in the case of potassium and calcium were explained as due to the alteration in composition which the ocean has undergone since the Protovertebrate form appeared, for not only has there been an increase since then in the saline concentration of the ocean water but there has obtained a change in the proportions of its basic constituents. This has been brought about by the continual elimination of the calcium and potassium and the retention of the sodium and in large part, of the magnesium derived from the river discharge from the land areas of the globe.

In 1904, in discussion of the history of the chemistry of the ocean, the author amplified these observations and evidence was advanced to show that this history fully accounted for the difference between the sea water of to-day and mammalian blood plasma on its inorganic side.

A very great difficulty which obtained in the way of definitely establishing the oceanic origin of the inorganic composition of the blood plasma was the fact that at the time there had been no analyses made of the inorganic constituents of the blood plasma of any Vertebrate other than mammalian. Even of the plasma of mammals only a few analyses were on record, and of these only those recently made were, with some reservations, acceptable as reliable. Amongst the latter are the analyses of the sera of the ox, pig and horse made by Bunge, and of the ox, sheep, goat, horse, pig, dog, rabbit and cat made by Abderhalden. Of the serum of man only the analyses of Carl Schmidt, made in 1850, are on record, but although in making these he used the best of the methods of separation and estimation of the elements then in use, methods which, however, would not pass muster to-day, his results do not tally with those which have been obtained by the

writer in the analyses of specimens of blood serum from a number of normal human subjects. Schmidt's estimates of sodium and potassium exceed what may be found by careful present day methods by at least 10 per cent and 40 per cent respectively.

While there is in the analyses of Bunge and Abderhalden a marked similarity of proportions in the potassium, calcium and magnesium in relation to the sodium, it was open to question whether these proportions would be found to obtain in the blood plasma of birds, reptiles, amphibia and fishes. One could not, therefore, make a generalization for the whole of the Vertebrate class from what was found in a few of the most highly developed.

A similar difficulty obtained also in the case of the blood plasma of Invertebrates. There were on record indeed a number of analyses of it in different forms, but they did not give concordant results even for the same form. There were those of Genth (1852), Gotch and Laws, (1884) and McGuigan (1907) on the blood plasma of the horseshoe crab, *Limulus polyphemus*, whose habitat has been marine ever since the early Cambrian period. While the discrepancies in these are in some cases at least extraordinarily great, Genth's results, even as regards the composition of the blood of horseshoe crabs from two different points in Chesapeake Bay are quite unlike and unexplainable except on one of two assumptions, either that his methods of analysis were imperfect or that the relative proportions of the inorganic elements in the blood of this form are very inconstant. That his methods did not give exact results may be shown by the fact that the percentages of potassium he found in samples of blood from horseshoe crabs of neighbouring localities in Chesapeake Bay were widely different, the element appearing to be twice as abundant relatively in the one case as in the other. Discrepancies of a certain degree may be explained, for the methods employed in such analyses, and especially for potassium, seventy-five years ago were much less exact than those of to-day, but even a defective method should give, under like conditions, uniform results.

The differences between the results of McGuigan on the one hand and those of Gotch and Laws on the other, would, if these represented actual variations in the composition, make it impossible to regard the blood in *Limulus* as uniform either relatively or absolutely in its inorganic composition, after a life in the sea almost coextensive with geological history.

How far the results of the analyses of the blood of *Limulus* made by

Gotch and Laws compare with those of Genth and McGuigan may be gathered from the following summary of the relative proportions derived from the results of their analyses:

	Na	K	Ca	Mg	SO_3	Cl
Genth (a)	100	12.4	5.49	7.97	8.87	170.3
Genth (b)	100	6.11	4.86	11.06	8.56	161.5
McGuigan	100	4.72	5.51	10.29	4.83	168.5
Gotch and Laws	100	5.30	3.83	11.67	7.65	157.8

That the osmotic pressure in the sea water to a certain extent influences the osmotic pressure of the blood plasma of marine forms has been demonstrated by Dakin (1908), Bottazzi (1897), Rodier (1899) and others, but the determinations indicating the influence were made by the cryoscopic method, and they do not show that the relative proportions of the inorganic constituents are changed, as the results may be explained by parallel changes in the concentrations of all these constituents. Except in the case of *Limulus* it is a question whether the changes in osmotic pressure of the blood plasma demonstrated as occurring with changes in that of the external medium are due in some cases to alterations even in the concentrations of the inorganic constituents. As will be indicated more fully later, the Δ of the blood plasma of the dog fish, *Acanthias vulgaris*, caught off the Atlantic Coast of Canada, where the ocean water has a Δ ranging from $-2.0°$ to $-2.3°$C., was found to be $-2.035°$C. while that of the salts of the same was only $-1.075°$C., the difference between these two pressures being due to urea and ammonia salts present in the blood plasma.

Of the remaining analyses, on record, of the inorganic constituents of the blood plasma of other Invertebrates there are those of Griffith (1892) on the blood in a number of Crustacea, including the lobster, *Homarus vulgaris*, and of a number of Mollusca including the Cephalopods, *Sepia officinalis* and *Octopus vulgaris*. How far these analyses are representative one is unable to determine for his results have not been checked hitherto, but the inorganic composition of the blood in the lobster, *Homarus vulgaris*, as Griffith has determined it, differs so markedly and irregularly from what the writer found it to be in *Homarus americanus* that it is necessary to hold in suspense any opinion as to their acceptability. As he does not indicate the methods he employed one is at a loss to account for the results he obtained in these analyses.

In 1907–1910 the writer, recognizing that in order to fully prove the

oceanic origin of the inorganic portion of the blood plasma in Vertebrates and Invertebrates it was necessary to extend our knowledge of the organic composition of the blood plasma in the lower Vertebrates and in some of the marine Invertebrates, and to attain this knowledge he undertook the analyses of the blood plasma of two Elasmobranchs, *Acanthias vulgaris* and *Carcharias littoralis*, of two Teleosts, *Gadus callarius* (the cod) and *Pollachius virens* (the pollock) and of the horseshoe crab, *Limulus polyphemus*, found at Woods Hole, Massachusetts, and the common lobster of the Atlantic Coast of Canada, *Homarus americanus*.

The results obtained in these determinations were published in 1910 and their significance was then discussed. The concentrations of the salts do not correspond for they varied in percentage from 1.282 and 1.293 in the blood plasma of the cod and pollock to 1.775, 2.852 and 2.982 in *Acanthias vulgaris*, *Homarus americanus* and *Limulus polyphemus* respectively, while the saline concentration of the sea water from which these forms were taken ranges from 2.60 to 3.165.[1] These values in themselves revealed nothing which explained the cause of the variations in concentration, nor did the relative amounts of the different salts in each salinity furnish a clue as to their origin. When, however, the porportions of the different elements in relation to sodium (Na = 100) in the salts of the blood plasma of each of these forms were placed in series side by side there was revealed at once a solution of all the problems involved in these variations. In the ratios as well as in the saline concentrations exemplified in all, except *Limulus*, it was unmistakably indicated that the blood plasma in each case, so far as its inorganic composition is concerned, is but modified sea water of the geological period in which the earliest representatives of the forms developed in the sea or took to a marine habitat. These concentrations are then paleo-oceanic and the ratios are *paleochemical* in character. In *Limulus* the concentration (2.982 per cent) and the ratios were found to be surprisingly like those of sea water of today. It and its ancestral prototypes have been oceanic as far back in geological history as the early part of the Cambrian period, and the concentration and ratios (e.g., that of magnesium) have changed as those of the sea water have increased and changed in the long ages since that period. The blood plasma of *Limulus* is, then, but the very slightly modified sea water of today. It is, therefore, neo-oceanic and its inorganic composition

[1] This variation is due to river discharge on the coasts of Nova Scotia, New Brunswick and New England.

may then be regarded as *neo-chemical* in character. Parallel to the concentration and ratios in *Limulus* are those in the tissue juice of the Medusæ,[2] *Aurelia flavidula* and *Cyanea arctica*, the ancestral forms of which have probably always been marine, and the tissue juice in these may be regarded as neo-oceanic in origin as well as *neo-chemical* in character.

Since 1910 the author has endeavoured to increase our knowledge of the exact inorganic composition of the blood plasma in a number of Vertebrates (frog, turtle and hen) not previously analyzed, but owing to the small quantities of serum which were ordinarily available for these analyses the results cannot be regarded as absolutely reliable, except perhaps in the case of the frog, and he consequently holds them in reserve until they have been confirmed by determinations made on larger quantities. He has analyzed also the blood plasma of dogs and of man a large number of times, using considerable quantities of plasma in these estimations in order to minimize inaccuracies in the results. The values obtained in the blood plasma of the dog were somewhat less than those obtained by Abderhalden, while those determined for the blood plasma of man were considerably less than those of Carl Schmidt (1850) of seventy-five years ago, the only ones for the plasma of man hitherto on record. Furthermore, as there must be "threshold" values for the elements in the plasma of Vertebrates, more especially for sodium and potassium, which are constantly being set free in the plasma from disintegrating old erythrocytes, he has, in order to ascertain the basic ratios of the elements for comparison with those of the "threshold" class, determined the concentrations of sodium, potassium, calcium, magnesium and chlorine in hydrocele and ascitic fluids from a number of human subjects and in the lymph of the thoracic duct of the dog and has obtained values from which one may elicit the more exact paleochemical ratios of man and the dog than can be derived from the "threshold" values of the blood plasma in the same.

With all the data available from the analyses referred to above it is now possible to discuss more definitely their significance in the interpretation of the origin of the fixed relations of the inorganic elements in the blood plasma of Invertebrates and Vertebrates.

IV. NEO-CHEMICAL BLOOD PLASMAS AND TISSUE FLUIDS. As indicated above, the blood serum of *Limulus polyphemus* is remarkably like

[2] A Medusa (*Limnocnida Tanganyikae*) occurs in the fresh water Lake Tanganyika of Eastern Central Africa. This may be a descendant of a form which had its habitat in the lake when it was directly and freely connected with the ocean.

in its inorganic composition, that of the ocean water of its habitat and in this respect it is parallel to the tissue fluid that may be obtained from the liquefaction of the disc of *Aurelia flavidula*. The averages of the percentages of the elements obtained in three series of determinations and the ratios, to compare with those found in *Aurelia* and ocean water, are as follows:

TABLE 1

	Na	K	Ca	Mg	SO_3	Cl
Limulus, concentration per cent	0.8885	0.04989	0.03613	0.09955	0.1184	1.6608
Ratios	100	5.62	4.06	11.20	13.39	186.9
Aurelia flavidula ratios	100	5.18	4.13	11.43	13.18	185.5
Ocean water ratios (Dittmar)*	100	3.613	3.911	12.10	20.9	180.9

* Calculated from Dittmar's analyses (1884), *Challenger Report, Physics and Chemistry*, Vol. I.

The total salts in the blood plasma of *Limulus* amounted to 2.982 per cent, whereas in the ocean water of the habitat of this form the salts ranged from 2.90 to 3.12 per cent. The Δ corresponding to the concentration of 2.982 per cent was ascertained to be -1.875°C., while that of the fresh blood plasma was found to be -2.04°C. What accounts for the difference between the two (-0.165°C.) was not ascertained but it may possibly be explained as due to the presence in the plasma of urea and other metabolites.

Limulus has been a marine form since its origin as far back, at the latest, as the close of the Cambrian period, during which long range of time just as the concentration and the ratios of the inorganic elements in the ocean increased and altered, so altered also, and in a parallel fashion, except in one detail, the concentration and ratios of the same elements in its blood. The exception is in respect to the sulphates (SO_3), the ratio of which in the plasma of *Limulus*, as also in liquefied disc of *Aurelia* is less than two-thirds of that in sea water, but considering the parallelism in the ratios of the other elements, it is a minor variation.

V. Paleochemical blood plasmas. The concentrations of the salts in the blood sera of the forms now to be discussed vary very considerably, ranging from 0.6335 in the blood plasma of the frog, *Rana*

virescens, to 2.852 per cent in that of the lobster, *Homarus americanus*, and in the non-marine forms they have been found to be less than 0.95, while in the marine forms they exceed 1.28 per cent. The determinations obtained by analyses are given in percentages of the sera in Table 2.

TABLE 2

	Na	K	Ca	Mg	Cl	SO_3
Homarus americanus (Lobster)	0.903	0.0337	0.0438	0.0156	1.547	0.0604
Acanthias vulgaris (Dog fish)	0.5918	0.02739	0.01609	0.0146	0.9819	
Carcharias littoralis (Sand shark)	0.6173	0.0355	0.0184	0.0172	1.042	
Gadus callarias (Cod)	0.416	0.0395	0.0163	0.00589	0.6221	
Pollachius virens (Pollock)	0.4145	0.01797	0.01286	0.00608	0.5613	
Rana virescens (Frog)	0.1975	0.0233	0.00627	0.00155	0.2679	
Dog (Lymph from thoracic duct)*	0.3033	0.0201	0.0085	0.0023	0.4231	
Man: Serum†	0.302	0.0204	0.0094	0.0021	0.389	
Pleuritic fluid‡	0.2956	0.02095	0.00839	0.0021	0.3425	
Hydrocele fluid§	0.3023	0.01497	0.00842	0.001914	0.3737	
Ascitic fluid	0.2935	0.0164	0.0091	0.00184	0.3888	

* Average of the results from four different samples.

† Average of the results from the sera of four adults.

‡ Pleuritic fluid analyzed was tinged with hemoglobin and its concentration in potassium in excess of that in either the ascitic or hydrocele fluids may thus be explained.

§ Average of the results from the hydrocele fluids of eight adults.

The ratios of the elements determined from these, compared with those found in seawater are as given in Table 3.

Inspection of the results of the analyses given in table 2 reveals wide differences in the percentage concentrations of the elements in the sera of the different forms listed. The total salts in each case differ. In the lobster, the dogfish, cod and pollock, all marine forms, they amount to 2.852, 1.7739, 1,283, and 1.2934 per cent, respectively, while in the frog, dog and man they total 0.6335, 0.952, and 0.895 per cent. The concentrations in the plasmas and the sera of the forms named, as compared with that of the sea water, are in percentage: lobster, 95; dogfish, 59; cod and pollock, 44; while in the sera of the frog and man they are 21 and 30, on the basis of 3 per cent as the average saline concentration of the sea water in the immediate vicinity of the coast from Massachu-

setts northward to Cape Breton Island. So widely, accordingly, do these values vary that it is impossible from them alone to obtain an interpretation of their origin.

TABLE 3

	Na	K	Ca	Mg	Cl	SO_3
Ocean water (Dittmar)....	100	3.613	3.911	12.10	180.9	20.9
Homarus americanus.....	100	3.73	4.85	1.72	171.2	6.67
Acanthias vulgaris.....	100	4.61	2.71	2.46	165.7	
Carcharias littoralis.....	100	5.75	2.98	2.76	168.8	
Gadus callarias.....	100	9.50	3.93	1.41	149.7	
Pollachius virens.....	100	4.33	3.10	1.46	137.8	
Rana virescens.....	100	11.8	3.17	0.79	135.6	
Dog.....	100	6.62	2.8	0.758	139.5	
Man: serum.....	100	6.75	3.10	0.695	128.8	
Pleuritic fluid.....	100	7.08	2.83	0.71	115.8	
Hydrocele fluid.....	100	4.95	2.78	0.666	123.6	
Ascitic fluid.....	100	5.58	3.10	0.626	132.4	

When, however, the ratios of the elements sodium, potassium, calcium and magnesium in the sera of these forms, as given in table 3, are examined there is revealed a degree of parallelism between the series from each of the forms which points unmistakeably to a common origin for all. Summarily these sera are, so far as their inorganic composition is concerned, but sea waters of different concentrations, sea waters, not of to-day, but as an attempt will be made to show, sea waters each derived from a particular period in the remote past history of the ocean and, in the marine forms, affected in each case by the change in composition and increase in concentration of the salts which have taken place in ocean water during the long time intervening between the age of origin in each case and the present period.

The parallelism in the series of ratios is approximate, not absolute, though significant. In two of these series, namely those of the cod and the frog, the ratios for potassium greatly exceed what they are in the other series. The quantity of serum of the cod which was used in the analyses was tinged red, due to a slight laking of the red corpuscles which in the cod are rich in salts of potassium. In the serum of the closely related form, the pollock, the ratio is less than half of what was found in the cod. To laking of the red corpuscles in the blood of the frog must be attributed the high ratio for potassium found in the serum of this form. The red corpuscles of the frog contain 0.200 per cent of

potassium. The quantity of serum used for analysis was derived from the blood of frogs kept in the laboratory over the winter to the spring and it was, as it separated from the clot, distinctly tinged red, this indicating laking of the red corpuscles. Indeed it is difficult to obtain serum untinged with hemoglobin from frogs kept in the laboratory through the winter, the Δ of the serum of which is -0.4°C. as compared with the $\Delta = -0.44$°C. of the serum of the frog in September. The excess in the ratio for potassium in the serum of the frog over that found in the sera of the other forms may be thus explained.

The ratios for chlorine vary within wide limits. That they should do so would follow from the interchangeability of the Cl, PO_4, SO_4, CO_3 and like anionic groups which obtain in varying concentrations in the blood plasma of Vertebrates as products of metabolism, and to a certain extent as the result of exchange between the inorganic constituents of the red corpuscles and the plasma in the maintenance of the acid-base equilibrium. The degree of interchangeability attained does not depend on the concentrations of the elements sodium, potassium, calcium and magnesium, whether these are high, as in *Homarus*, or low, as in the frog and mammals. Only a certain amount of the chlorine is so affected, and it may, in the blood serum of the Selachians, approximate that in the serum of mammals. A decrease of more than 20 per cent in the chlorine in a serum in which the salts total 0.9 per cent may exceed in amount the decrease in the serum of the *Homarus* in which the salts total 2.852 per cent. The concentration of chlorine must, therefore, vary not only absolutely but relatively in the sera of *Homarus* and Vertebrates, whatever the concentrations and ratios may be of the sodium, potassium, calcium and magnesium, which do not, so far as we know, interchange in the slightest degree physiologically in the body fluids and tissues.

The high concentration of the salts, 2.852 per cent, in the serum of the lobster would appear to indicate that it is of neochemical rather than of paleochemical origin. The Δ to correspond to this concentration was found to be -1.735°C. while the sea water at the bottom of the Bay (at St. Andrews, New Brunswick) where the lobsters from which the serum was analyzed were obtained, gave a Δ of -1.76°, but the water of the surface at the same point gave -1.635°C, and, therefore, the blood serum of the lobster, on its inorganic side, would appear to be but modern sea water. That it is not, however, is indicated by its low content in magnesium which is less than 15 per cent of the magnesium of the sea water of the locality. The ratios for magnesium

are respectively 1.72 and 12.0. The sulphates (SO_3) in the blood are less than one-third of the sulphates of the sea water. There is, therefore, in regard to the magnesium and the sulphates a selective action which keeps these constituents of the blood, in spite of the osmotic pressure which those elements in the sea water exert, at a low level, a selective action which after millions of years in a marine habitat cannot be regarded as a recently acquired character, and yet is still effective.

This selective action and the series of ratios of the inorganic elements in the blood serum of the lobster which are practically parallel with the series of ratios of the same elements in the blood sera of Selachians and Teleosts compel one to regard the lobster's serum, in its inorganic relations, as merely more concentrated than such sera, just as the serum of the dogfish, with its saline content of 1.7739 per cent, is but a more concentrated Teleost serum. The concentration, aside from the fact that it is caused by the osmotic pressure of the ocean water, does not signify, while the ratios distinctly indicate, as do those of the sera of Selachians and Teleosts, that they are not those found in the sea water of to-day. The serum of the lobster thus differs markedly from the serum of *Limulus*.

The marine history of the lobster can be traced as far back as the Jurassic period. The ancestors of the *Homaridae* are supposed to have been fresh water forms of an Astacoid character and the fossil remains of a species of *Astacus*, *A. vectens*, have been found in the so-called Lobster Beds in the Greensands of the Isle of Wight, belonging to the Cretaceous period. In the earlier period, the Jurassic, the Macrouran Decapods arose and remains of these are found in deposits formed more or less in still waters, such as the fine grained Solenhofen slates, in which they are mingled with remains of terrestrial and fresh water forms. This suggests that the Mesozoic ancestor of the lobster of to-day must either have been of a fresh water type or one that resorted to embayments, lagoons or stretches of water more or less surrounded by land and, therefore, fresh or brackish in character.

A present day representative, of fresh water habitat, of the Jurassic ancestor of the *Homaridae* is the crayfish, *Astacus fluviatilis*. The concentration of the salts in its blood has been determined, but very indefinitely. Witting (1858) found it to be 1.55 per cent. He analyzed the total ash but the results cannot be accepted to-day for it is evident that in the incineration of the dry serum residue to obtain the ash a very considerable amount of the chlorine was lost, and further, the methods he employed, those of his time for the estimation of sodium

and potassium, were very inexact. Later determinations by Halliburton (1885), Fredericq (1899) and Quinton (1900), dealt only with the soluble portion (chlorides chiefly) of the ash of the blood, and their results indicate a range from 1.13 to 1.2 per cent, while the range of the Δ which Fredericq ascertained was from 0.765° to −0.83°, which, if wholly due to the inorganic salts, would postulate for these a concentration of 1.31 to 1.5 per cent, about half of that in the blood of the lobster.

If in the Astacoid ancestral form of the lobster of the Cretaceous period the saline concentration of the serum was the same as that of *Astacus fluviatilis* of to-day, which is extremely likely, then an increase in concentration from 1.4 per cent to 2.85 per cent in the serum of the lobster has developed, through the osmotic pressure of its marine habitat, in the long interval between the Cretaceous and the present period. How long this was it is difficult to establish, but the minimum length estimated is 5,000,000 years, while Lord Rayleigh (1910), basing his observations on the amount of helium contained in a sample of hematite from Antrim County, Ireland, derived from strata of the Eocene, and on the amount of this gas liberated in a measured time from a certain quantity of uranium, suggested that at least 30,000,000 years have passed since the Eocene, the period succeeding the Cretaceous. Whether, therefore, we accept the maximal or the minimal estimate, the interval is of great length and in all those millions of years the paleo-ratios have been maintained in the blood of the lobster.

The concentrations of the salts in the serum of the sand shark, dogfish, cod and pollock were found to be 1.938, 1.773, 1.283 and 1.293 per cent respectively, that is, ranging from less than 64 and 59 per cent in the two former to less than 43 per cent in the two latter, of the concentration in sea water. The differences in this respect between the two groups is undoubtedly due to the difference in the lengths of time during which the Elasmobranchs and Teleosts have been marine in habitat. The Elasmobranchs have always been marine forms since their origin,[3] which was, at the latest, Devonian, and, if the Ostracoderms are Protoelasmobranchs, probably Ordovician, but the Teleosts originated in the Jurassic and, it is supposed, from an *Amia*-like Ganoid. The Ganoids were abundant in the Paleozoic and Mesozoic periods, but they were probably fresh water forms, although, through the scanti-

[3] A shark, *Carcharias nicaraguensis*, occurs in the fresh water Lake Nicaragua, to which access from the sea by this shark is readily possible by the river outlet of the lake.

ness of fresh water deposits, no fossil remains of them indicating a fresh water habitat are known. The present day Ganoids, including *Amia*, are fresh water forms, and, therefore, the Ganoid ancestor of the Teleosts, which to-day are fresh water and marine forms, was, in all probability, a fresh water form, or one which occurred occasionally in brackish water, as does the sturgeon of to-day.

The higher concentration of salts in the dogfish and sand shark can be attributed to the action of the osmotic pressure of the ocean water exercised for a much longer time than was the case with the Teleosts. The interval between the beginning of the Ordovician and the beginning of the Jurassic was very much, possibly three to four times, greater than that between the Jurassic and the present period if one can accept the general opinion of geologists on this point.

In all the time which has passed since Eovertebrates appeared, the steadily increasing salinity of the ocean, although it has increased the concentration of the salts in the serum of the dog fish and sand shark, has not, as it has done in the serum of the lobster, brought the concentration to that of their present habitat. The concentration in the serum of the dogfish is 1.7739 per cent as against more than 3 per cent in the ocean. If the concentration in the ocean water of the Ordovician and Silurian periods, when Protoelasmobranchs obtained, was approximately 1 per cent, then it has since increased by 200 per cent, but in the serum of Elasmobranchs only by at most 80 per cent.

In that long interval also, the paleo-ratios were maintained. The ratios for magnesium in the serum of the dogfish and sand shark are 2.46 and 2.76, both being higher than the ratios in the serum of the cod (1.46) and pollock (1.41), all in marked contrast with that of the magnesium in the ocean water of today (12.1).

The osmotic pressure in the serum of the dogfish, as already stated, indicated by the Δ, is $-2.035°$C. but the total salts of the same give a Δ of $-1.073°$C. only. The difference between these is due to urea and salts of ammonia, which thus assist in balancing the osmotic pressure of the serum against that of the sea water of the habitat of this form. The urea in the serum, as the author found, was 2.026 per cent, which would give a Δ of $-0.63°$C., leaving a Δ of $-0.332°$C., due to salts of ammonia, for the amount of ammonia present in the serum was found to be 0.1727 per cent, a concentration slightly greater than N/10 and sufficient to account for it.

The extraordinary concentration of urea in the blood of Elasmobranchs is a matter of record. Staedeler and Frerichs, in 1858, noted

its presence in "colossale Quantitäten" not only in the blood but also in the muscles, liver, kidney, spleen, pancreas, ovaries and testes of sharks, and obtained two ounces of it from the liver of *Scyllium canicula*. Von Schroeder, in 1890, found the blood, as a whole, of *Scyllium catulus* contained 2.6 per cent of urea, and he estimated that the plasma must contain it to the extent of 3.1 per cent. Rodier, in 1899, noted that one-third of the osmotic pressure of the blood of all the shark forms he examined, and which he found to correspond to a Δ of -2.05°C. was due to urea, which, consequently, must have been present to the extent of more than 2 per cent, assuming that a gram-molecular solution of urea gives a Δ of -1.85°C.

That ammonia is always present in considerable amounts in the serum of Selachians generally would follow from Erwin Herter's observations on the urine of *Scyllium catulus* which he found to be rich in ammonia and P_2O_5 and markedly acid.

The low concentration of salts in the blood, as compared with the concentration of salts in sea water, and the maintenance of the paleoratios, in Selachians, after very many millions, possibly hundreds of millions of years of life in the sea, indicate unmistakably how inflexibly constant, practically, is the action of the organ concerned, the kidney, in the Vertebrates.

In the serum of the frog the salts amount to 0.633 per cent, and account for the total Δ (-0.395°$-$$-0.40$°C.). This concentration is the lowest yet found in Vertebrates, the next lowest possibly being that of the serum of *Tinca vulgaris*, the tench, a fresh water fish, which, according to Dekhuyzen, gives a Δ of -0.46°C., which, if wholly due to salts, would postulate a concentration of approximately 0.73 per cent. The concentration ranges between 0.8 and 0.9 per cent in birds and reptiles, and it is in the serum of the dog and man 0.952 and 0.895 per cent. The ratios for the serum of the frog, with the exception of that for potassium, which is high, and, as already explained, due to laking of the corpuscles when the serum analyzed was obtained, are parallel with those found in the serum of the dog and man.

The Amphibia first made their appearance, so far as is yet known, early in the Carboniferous period, and were then chiefly, if not wholly, fresh water and land forms. The positions in rock formations in which the fossil remains of some of these (e.g., *Dendrerpeton*) have been found indicate a burrowing habit, and, consequently, they were land forms in adult life, as in the case with the great majority of the Amphibia of to-day, all of which, however, pass their earlier life in fresh water.

Even in the adult stages, these resort to fresh water for more or less lengthy periods each year. Fossil remains of *Rana* have been found in the Eocene rocks and its ancestry must have extended, with a life in similar habitats, back through the Mesozoic to the Carboniferous. Such habitats, and especially that in fresh water, would diminish the concentration of the salts in the serum of the frog, just as the marine habitat has increased the concentration of the salts in the serum of the Selachians above what it must have been in the Ordovician,[4] and above what it must have been in the Ganoid ancestors of the Teleosts in the Devonian. The low concentration in the serum of the frog must thus have developed.

What was the original concentration?

The reptiles first appeared in the Carboniferous and were undoubtedly derived from amphibian forms. They were the dominant forms in the Mesozoic and from them, before the close of that period, arose the first avian and mammalian forms. These are land forms only, except the Cetacea, and their habitat could not, therefore, influence to any degree the concentration of the salts in their serum. Even in the Cetacea, which had their origin in the Cretaceous from a form which was, very probably, also the ancestor of the Ungulata, the inorganic composition of the blood, after millions of years of marine life, as will be shown later, has been maintained remarkably constant. The fact that the concentration of salts in the serum of birds and mammals ranges between 0.8 and 0.95 per cent indicates very strongly that the original concentration was approximately 0.9 per cent which would first be established in the Cambrian when, probably, the Protovertebrates first appeared. The transmission of this concentration to the birds and mammals which arose first in the late Mesozoic was unaffected by their non-marine environment. With this concentration there were transmitted also the ratios of the sodium, potassium, calcium and magnesium found in the blood serum of the higher Vertebrates which is, therefore, from its inorganic side, but the sea water of a remote geological age.

VI. The organ concerned in maintaining the paleo-ratios. How far back in geological time was the age when the concentration of the salts in the ocean was less than 1 per cent, it is difficult to determine exactly. It must have been earlier than the most ancient fossil

[4] The recently reported discovery of fish scales of a form, *Eoicthys Howelli*, in the Cambrian rocks of Vermont, indicates a much earlier origin for fishes than was hitherto acknowledged by Paleontologists.

remains, so far known, indicate, that is, in the early Cambrian or late Proterozoic for Protovertebrates or Eovertebrates must have long preceded the Vertebrates to which they gave origin. If it were known even when the Vertebrates first appeared there would remain the difficulty of determining approximately the length of the time passed since then. This is necessarily so for the estimates of the age of the earth vary very widely. Sollas (1909), basing his determinations on geological data alone, gives the minimal age as 80 million years, and the maximal as 175 million. Joly, who, in 1899, using as data the amount of sodium now in the ocean and that found in the annual river discharge of the globe, estimated the age of the ocean as 89 million years, now concedes it may be as much as 175 million (1925). On the uranium-lead ratio found in minerals from the pre-Cambrian and on the time required for the conversion of a given amount of uranium to a given amount of lead, are based estimates which range from 560 to 1340 millions of years. Lord Rayleigh (1911), from the helium content of a specimen of the mineral sphene from Archean strata estimated that about 710 million years have passed since it was formed.

These estimates indicate how uncertain is the length of geological time, and, therefore, also that of the age of the ocean. We are, nevertheless, justified in assuming, until more decisive determinations are made, that it is at least in excess of the minimal estimate, or about 100 million years. It would follow, then, that as the saline concentration of the ocean is now more than three times what it must have been when the concentration became fixed in the blood plasma of the earliest Vertebrates, more than 60 million years have passed since Eovertebrates arose. This enormously long period, during which the blood plasma of the higher Vertebrates has been reproducing paleo-oceanic conditions in the concentration of its salt and in the ratios of the sodium, potassium, calcium and magnesium in it, emphasizes the importance, in one respect, of the organ which has maintained through that long time this concentration and these ratios practically unchanged.

That this organ is the kidney would appear to be unquestionable. In the higher Vertebrates the skin and the intestinal mucosa must, as it would appear, play but a very negligible part in the elimination of sodium, potassium, calcium and magnesium from the blood plasma, while these elements obtain in the urine in quantities which imperatively postulate that the kidneys are concerned in their elimination from the blood plasma so as to render certain a constancy of concentration in it. Even in the lower Vertebrates the intestinal mucosa and the gills

must be negligible as factors concerned in maintaining this constancy. Erwin Herter found in the urine of *Scyllium catulus* 0.0415 per cent calcium and 0.1416 per cent magnesium, while the sea water of the locality (Naples) from which the animal was taken contained 0.0464 per cent calcium and 0.1421 per cent magnesium. He did not determine the total amounts of the sodium and potassium in the urine, but the amount of chlorine he found was 1.3543 per cent, whereas in the sea water of Naples it was 2.1142 per cent. This discrepancy may readily be explained, for the hydrochloric acid in the gastric juice of *Scyllium catulus*, according to Richet (1878), ranges in amount from 0.69 to 1.29 per cent and as the acid of the gastric juice is neutralized in the intestinal tract, its chlorine may pass, as chlorides, out with the intestinal excreta, while its place in the urine is taken by phosphoric acid, which is exceedingly abundant in the urine of this form. The very close correspondence between the sea water and the urine as regards the amounts of calcium and magnesium they contain indicates very clearly that the intestinal mucosa and, perhaps, also the gills, have no selective action on the constituents of the sea water, which, therefore, as such, unchanged, diffuses into the blood stream of the circulation in Elasmobranchs.

There are in Invertebrates no structure or structures having a function or functions quite similar to those of the Vertebrate kidney. There are in *Limulus* the coxal glands which are excretory, but they do not control the inorganic composition of the blood plasma, except as regards SO_3, and to a very negligible degree as regards magnesium, while the concentration of the salts is the same as that of the habitat. In the blood plasma of the lobster the paleo-ratios are maintained, and in this function the glands are like the Vertebrate kidney, but the concentration of the salts is that of the habitat of this form. As indicated already, the blood plasma of the Astacoid ancestral form of the *Homaridae*, of the Cretaceous, had probably a saline concentration approximately that of the blood plasma of the crayfish, *Astacus fluviatilis*, a fresh water form of today. A marine habitat, extending in time back to the Cretaceous period, has caused the total loss of the power of the coxal glands in the lobster to control the saline concentration, while that of maintaining the paleo-ratios has been retained.

The control over the concentration, even in Vertebrates, is not absolute, as shown by the changes in osmotic pressure of the blood plasma in fishes which pass from fresh water to salt water, or from sea water of low to sea water of high saline concentration. Dakin (1908) has

ascertained that the Δ of the blood of a fresh water eel was −0.57°C, while that of eels taken at the Helgoland was −0.635°C. He found the blood in specimens of the plaice, *Pleuronectes platessa*, taken at Kiel, where the water is brackish and gives a Δ of −1.09°C., to give a Δ of −0.665°C., while in specimens taken at Helgoland, where the sea water gives a Δ of −1.9°C., the Δ was 0.787°C. The Δ of the blood therefore increased 19 per cent. In *Gadus morrhua*, however, it was −0.73°C. in sea water, with a Δ of −1.2°C., but in specimens from the North Sea, which had a Δ of −1.9°C., it was −0.757, an increase of only 3.9 per cent. Loeb and Wasteneys (1915) found the tissue juices of mud minnows, *Fundulus heteroclitus*, which in sea water of a Δ of −2.071°C. give a Δ of −0.85°C., after eight days in water with a Δ of about −0.16°C. gave a Δ of −0.77°C. It is evident, therefore, that the concentration may increase within a more or less narrow limit, but as the author's analyses of the blood of the pollock and cod show, the paleo-ratios would be maintained.

This suggests that the function of controlling the concentration and that of maintaining the paleo-ratios are independently performed. That they do not wholly overlap is indicated by the saline concentration and the paleo-ratios in the blood plasma of the Elasmobranchs, which have been denizens of the sea since the Cambrian. In that long period the saline concentration, though it has increased from less than 1 per cent to 1.774 per cent, has not, as it has in *Limulus*, also a denizen of the sea since the Cambrian, attained that of the sea water of to-day, while the paleo-ratios have been maintained practically unchanged. This would indicate that the power of controlling the saline concentration is not as inflexibly exercised as is that which maintains the paleo-ratios.

However varying may be the efficiency with which the two functions are performed there can be no doubt about their primacy in the work of the kidney in Vertebrates. These functions are fundamental and are of more ancient origin than that of excreting the waste metabolites. The urea in the blood plasma of the higher Vertebrates is of low concentration. In that of man it ordinarily does not exceed 0.03 per cent. Salts of ammonia are present but only in traces, ranging, in estimates (Folin), from 0.00003 to 0.00008 per cent, reckoned as NH_3. In the blood plasma of the sharks the urea exceeds 2 per cent, and in that of the dogfish the ammonia, reckoned as NH_3, is 0.1727 per cent, or slightly more than N/10. The concentrations of these are, therefore, more than 600 and 3000 times, respectively, those of the

urea and ammonia in the blood plasma of man. Their occurrence in such concentrations in the blood plasma of sharks indicates how relatively inert in their elimination are the kidneys of these forms. The urea and salts of ammonia thus retained help to balance the osmotic pressure of the plasma against that of the ocean water, which equalization would be otherwise impossible since the kidneys in Elasmobranchs, which have had a marine habitat extending at least over 60 millions of years, still exercise a control over the concentration of the inorganic elements and thus prevent it from reaching that in sea water. This control of the concentration of the inorganic elements and the maintenance of the paleo-ratios, because of their inflexibility as it were, must be functions of an origin very much more ancient than that of the function of eliminating metabolites.

The functions of controlling the concentrations and of maintaining the paleo-ratios must have developed before the Protoelasmobranchs and even the Protovertebrates arose. The renal organ in the Eovertebrates consisted, as does the renal Anlage in all Vertebrates to-day, of three divisions, the pronephros, the mesonephros and the metanephros. The kidney in fishes and Amphibia is derived from the mesonephros, and that in reptiles, birds and mammals is developed from the metanephros. This would postulate that the function of controlling the inorganic composition of the blood must have been exemplified in the primitive kidney of the Eovertebrate.

In the long ages the kidney has ever thus performed functions which, for constancy and regularity, are unrivalled in the world of life, except by those of the cell nucleus, which, of course, is of vastly more remote origin. This constancy contrasts with the variations in functions which the other organs in Vertebrates have undergone. It has made the Vertebrates, with all their ranges of development, possible. Without such a constancy there could be no change in habitat from sea to land and fresh water and back again to sea, for with such a change there would be a variation in the inorganic composition of the internal medium, an impossible handicap in the struggle for existence, which would greatly affect the development of the organs after the Eovertebrate stage were passed.

As an example of this constancy maintained in a change of habitat the Cetacea may be cited. The whales arose in the Eocene and, as already stated, from a form which in the Cretaceous gave origin also to the Ungulata, according to some comparative anatomists, yet after a life in the sea almost as long as that of the *Homaridae* (lobsters) their

blood, although sea water has, through the intestinal mucosa, had constant access to it, has an inorganic composition very little different from that of the blood of their supposed collateral descendants, as indicated in the following table:

IN 100 GRAMS OF BLOOD	Na	K	Ca	Mg	Cl
*Balaena mysticetus** (The Right whale)	0.1805	0.2004	0.00646	0.00448	0.2685
Pig†	0.17859	0.1917	0.00485	0.00533	0.2690
Horse‡	0.19074	0.2273	0.00364	0.00384	0.2785

* Analyses by the author.
† Analyses by Abderhalden.
‡ Analyses by Abderhalden.

The approach to parallelism is remarkably close and the variations, such as they are, may be due to the red corpuscles which in the whale, as in the horse and pig, are rich in potassium but free from sodium, quite in contrast, in this respect, with the red corpuscles of the ox, sheep, goat, dog and cat, which are rich in sodium and poor in potassium. The inorganic composition of the blood plasma in *Balaena* has not been determined but from the values given for the whole blood it would appear to vary, if at all, only negligibly from that of the blood plasma of the pig. Whatever it may be, if the whale and the pig are descendants of a common ancestral form which obtained in the Cretaceous, the millions of years of marine life have apparently had little or no effect on the inorganic composition of the blood of Cetacea.

The Vertebrate kidney, therefore, by its control over the concentration of the inorganic elements in the blood plasma and by its maintenance therein of the paleo-ratios, has thus perpetuated in the blood of Vertebrates the ocean water of the early Cambrian, if not of the late Proterozoic. It stabilized the inorganic composition of the internal medium in Eovertebrates and thus rendered Vertebrates, in their wide range of development in all habitats, and in all that is thereby involved, possible. It must, therefore, be regarded as taking precedence in the body as a Vertebrate organ *par excellence*.

VII. THE PALEOCHEMISTRY OF THE OCEAN. To understand the origin of the concentration of the salts and the ratios of the elements in the blood plasma of Vertebrates, one must review all the facts from which we can gather, as far as possible, the history of the composition of ocean water.

Immediately before or just at the commencement of the Archean period, the temperature of the rock crust of the globe was so high that all the water of the present oceans was, as water vapour in the atmosphere, which, in consequence, had a pressure, according to Joly, 270 times, according to F. W. Clarke 247 times, what it is now. When the cooling of the rock crust and of the atmosphere had progressed until the temperature of the latter was, according to Joly, 370°C., the first condensation of water took place on the rock surface. Even with a pressure of 190 atmospheres at this temperature, he holds, condensation would occur and, as the pressure was much higher, would go on till the pressure fell below 190 atmospheres. There would result rapid evaporation, again and again, for the temperature of the rock surface at many points would be so high that the water would condense there only to boil away immediately. The water, reacting at such a high temperature with the hot rock material, would then contain, in low concentrations at first, constituents of the superficial part of the crust, salts of sodium, potassium, calcium, magnesium, iron and aluminium, but with repeated evaporation at points these would there accumulate and when the condensation at points ceased to boil away, that is, when the temperature of the rock crust and of the atmosphere had fallen to below 100°C., the oceans began to form, and to continue as such, in depressions of the surface which became deeper through the weight of these great bodies of water acting on the more or less plastic rock crust.

The salts contained in such oceans were chlorides almost wholly. If sulphates and carbonates were present in more than exceedingly nominal quantities, there would form the insoluble carbonate and the sulphate of lime, resulting in deposits of limestone and gypsum, but deposits of gypsum are very scanty in the pre-Cambrian, of which, however, the Grenville and Sudbury deposits, the most ancient sedimentary strata of geological time, of a thickness of 114,000 feet (Coleman), are formed chiefly of limestone. This was probably of detrital origin, for the high content of CO_2 in the condensations of water on the exposed rock crust would convert a large proportion of the calcium salts dissolved therefrom into the insoluble carbonate which would thus reach the primal ocean, to be sedimented as such.

The chlorides were those of sodium, potassium and calcium. The chlorides of magnesium, aluminium and iron derived, with those, from the hot rock crust by the action of superheated water, continued for perhaps hundreds of years, would on the repeated evaporation of each condensation of superheated water be converted into magnesia (MgO),

alumina (Al_2O_3) and ferric oxide (F_2O_3), the first of which is soluble only in 55,368 parts of hot and cold water, while the two latter are insoluble in water and even in dilute acids.

Later, through the action of the chlorides of sodium, potassium and calcium on magnesia, some of the latter would pass into solution as chloride but the amount so affected must have been comparatively small.

What was the total concentration of all these chlorides, even after a million years, it is not possible to determine even approximately, but we can form some estimates of the relative proportions of these salts in the ocean water in the earlier portions of this period. These are based on the elementary composition of the primal rock crust before and immediately after the first oceanic deposits were formed. What the composition was at the beginning of the Archean cannot with certainty be determined from the composition of the most ancient portions of the present rock crust, for from it has been removed not only the salts of the ocean of today, but also those in the sedimentary deposits and all the calcium and magnesium in the limestone and dolomite formations of all the geological periods. It is, however, possible to total the amounts, in per cent, of the sodium, potassium, calcium and magnesium in these and in the present rock crust to a depth of ten miles and thus obtain estimates of the amounts of these in the rocks of the earth's surface before the first condensation of water affected them. This Clarke and Washington (1922) have done, and the following percentages are taken from the results of their determinations:

Na	K	Ca	Mg
2.72	2.56	3.63	2.07

The ratios of these would be:

Na	K	Ca	Mg
100	94.1	133.4	76.1

If the decomposition of the hot rock crust under the action of superheated water gave, as the first yield, these as chlorides and some of them as carbonates, the magnesium chloride being, as indicated, except in very minute amounts, eliminated as magnesia (MgO), the sodium,

potassium and calcium would appear in the earliest ocean water in the proportions indicated, the potassium only very slightly less, relatively, than the sodium, while the calcium would be one-third again as much.

If the sodium, potassium and calcium were set free as chlorides from the mineral constituents of the rock crust, not in the proportions indicated, but in such as the physico-chemical conditions acting on the decomposable mother minerals determine, then the ratios would and must have varied from those which are indicated above. This is shown to-day in such analyses of the water shed from Archean rock regions as have been made. F. D. Adams found the water of Reindeer Lake in the Archean region west of Hudson Bay very much richer relatively in potassium than in sodium. This is the case also with the water of the Churchill River and of Saskatchewan River above its junction with the Big Stone River, which rivers drain rocky areas of the pre-Cambrian type. In the waters of the lakes of the Bavarian Highlands, Rachel See, Würm See and Ronig See, the potassium is at least twice in amount that of the sodium. Further, the calcium of the original rock crust must have been there as silicates, simple and complex, not as carbonate or chloride, and the quantity of it released and passed into the ocean water must then have been less, probably very considerably less than its ratio (133.4) would indicate, and especially since the condensations on the rock crust must have been charged with CO_2 which obtained in the atmosphere in a high concentration, as the very high temperature of the molten magma of the crust did not permit it to retain it, except possibly in traces, as carbonates. It is even possible that the calcium in the primal ocean water was not very greatly in excess of the magnesium because the silicates of the latter are more readily decomposable than those of calcium.

It is, therefore, very probable that in the primal oceans as they were forming the potassium present was in excess of the sodium, possibly twice as much, while the calcium, so abundant relatively to sodium and potassium in the primal rock crust, must have obtained much less abundantly relatively than the sodium, and the magnesium was present at first in an almost negligible concentration. The ratios of these then may be thus represented:

Na	K	Ca	Mg
100	100–250	10–?	0.01–0.1?

If we range these with those of the ocean water of today:

Na	K	Ca	Mg
100	3.61	3.91	12.1

we see a complete alteration in proportions which along with the increase in the concentration of the salts from 0.1 per cent (?) in the earliest ocean water to 3 per cent and more as it is in the ocean of today, indicates the extent of the change which has taken place during all the geological periods.

How was this brought about?

First, as to the increase in concentration of the saline constituents. The decomposition of the surface of the rock crust, begun with the first condensations of water, was continued on the surface of the continental areas and further amounts of the salts of sodium, potassium, calcium and magnesium were added to the original sea water. The rate of increase in the concentration must have been slow, for the action of the water must have at first affected the more easily decomposable material, leaving a product, the first detrital material, which, carried to the sea, constituted the first sedimentary strata. This detrital material and the exposed Archean areas have been, and are still, undergoing decomposition, yielding soluble salts carried by the wash from the continental areas to the sea.

The concentration then increased but the ratios of the sodium, potassium, calcium and magnesium began to change from the beginning of the Archean, those of sodium and magnesium to increase, but at different rates, and those of potassium and calcium to decrease, also unequally.

Of the sodium added to the sea, comparatively little has been removed. In the formation of sedimentary strata sodium chloride is included, but in the denudation of these it is returned to the sea in an amount more or less corresponding to that which is removed from the sea in the formation of new sedimentary strata. Sodium chloride is carried to the land in the rainfall but this is returned to the sea in the river discharge. In the formation of salt beds by evaporation of landlocked arms of the sea, sodium chloride was thus removed but the total amount of these deposits is infinitesimal as compared with the total amount in the sea. There was therefore throughout geological time a steady increase in the concentration of sodium in ocean water.

The salts of potassium are also carried to the land in rain water. Potassium manifests a marked capacity to unite with silicates of alumina to form more or less insoluble compounds and these obtain whenever potassium salts come in contact with argillaceous material, the clays for example. This capacity to extract the potassium is increased if the silicates are mixed with organic matter, as in the soils holding decaying vegetable remains. Consequently all the potassium which rain water brings to the land surface is not returned to the sea in the river discharge. The potassium in the sea is taken up by the plankton organisms (Foraminifera, for example) which on dying fall to the sea bottom and, their remains decomposing, the potassium they contain, influenced by the decomposing organic matter, reacts with the argillaceous matter on which they rest to form the mineral glauconite, in which the potassium ranges, according to analyses made by Murray and Renaud (1884) from 2.09 to 3.5 per cent, according to determinations made by Roth (1879) from 2.8 to 7.3 per cent. It is consequently richer in this element than was the original crust of the globe. The formation of glauconite has continuously obtained throughout all the geological periods since the pre-Cambrian, for it is found in the primary formations of Russia and Sweden, in the sands and gravels of the Cambrian sandstone of North America, in the Ordovician of Quebec, and in the course Silurian sands of Bohemia. In the Mesozoic period it was more abundantly formed, and its deposits are very considerable in the strata of the Cretaceous. It is found in all Tertiary and post-Tertiary strata as it is to-day on all the continental shelves fringing the continents, where the sedimentary strata of the future are being formed, and it constitutes as much as, or more than, half of the deposits found in shallow waters.

We may postulate then that early in the pre-Cambrian (Archean) when soils holding vegetable matter did not exist and also before life began in the sea, the potassium concentration therein was increasing and very possibly at the same rate as the sodium, but when living organisms appeared, in the sea as plankton, and on the land surface as vegetable forms of a low order, possibly chiefly Protophytan, the rate of increase must have lessened, and, in consequence, the proportion of the potassium to the sodium became less and less. The high rate of increase of the sodium and the low rate of increase of the potassium have thus resulted in bringing the original ratio of these two elements (100:100–200) down to that in the sea water of today (100:3.613).

There has been a constant addition of calcium salts to the sea through-

out the geological periods and it has been, and is being, removed therefrom as calcium carbonate (limestone). The amount removed may be gauged by consideration of T. Mellard Reid's estimate of the total limestone in the sedimentary strata, according to which it is equivalent to a layer 528 feet thick completely enveloping the globe. The quantity of CO_2 which this mass contains must have been in the atmosphere of the pre-Cambrian period, as was also that from which the carbon of the coal and lignite of the Carboniferous and later ages was derived. This indicates that the concentration of carbon dioxide in the atmosphere was then much higher than it is now and it must have, in the earlier periods, prevented a relative increase in the concentration of the calcium in the sea water. With this large amount of carbon dioxide being slowly removed from the atmosphere, there would result, not at once, but ultimately, a slow gradual increase in the concentration of the calcium. We may thus explain the origin of the difference between the paleo-ratio 2.8 to 3.1 in the plasma of mammals and the neo-ratio 3.91 in the sea water of today.

The concentration of magnesium in the ocean water of the early pre-Cambrian, for reasons already stated, must have been infinitesimal, probably less than 0.005 per cent, and it is in the ocean water of today in excess of 0.10 per cent. There has, therefore, occurred an increase in concentration which has made it more than three times as abundant as the calcium. Magnesium goes in the river discharge to the sea as chloride and sulphate, and to a very limited extent also, as carbonate. Plankton organisms do not remove it from sea water, or if they do, it is in negligible quantities. When, however, these are eliminating the calcium, the latter, as carbonate, reacts with the magnesium chloride to form a certain amount of magnesium carbonate, which as such is included in the limestone deposits. The latter, in consequence, always contains this compound, and when it exceeds 10 per cent of a limestone deposit the latter is given the conventional name dolomite. Dolomites are found in the strata of all the periods down to and including the Cambrian. Magnesium, as carbonate, has thus been continually in the past removed from the sea but in amount much less than that of the calcium similarly eliminated, for its total in all the limestone and dolomite strata does not exceed 10 per cent of the calcium contained therein. Consequently, the amount of magnesium annually removed from the sea must fall far behind that of the calcium and it must, therefore, have steadily increased absolutely and relatively, though at a rate far behind that of the sodium. This

would account for the change from the paleo-ratio of 100:0.7–0.8 in the plasma of mammals to 100:12.1 in the sea water of today.

To summarize, throughout geological history the sodium in sea water has constantly increased, and at a high rate. The magnesium has increased very considerably, both absolutely and relatively, but at a slower rate than the sodium. The potassium has slowly increased absolutely, but decreased relatively, while the calcium has increased very slowly, both absolutely and relatively.

From the foregoing it may be concluded that the ocean water, when the Eovertebrates appeared in it, had a saline concentration of less than 1 per cent, and the ratios in it were:

Na	K	Ca	Mg
100	6.7	3.1	0.7

and that these ratios slowly developed from the earliest Archean when the saline concentration was very considerably less and the ratios very different, possibly, as already indicated:

Na	K	Ca	Mg
100	100–250	10?	0.01–0.1?

This was the time, without doubt, when living organisms, of the unicellular kind, made their appearance in the sea.

VIII. Possible paleochemical endowments in the animal cell. Life, it is generally assumed, originated in the ocean water of the Archean period, and the very earliest organisms must have been of the micellar or ultramicroscopic kind for the physical and chemical conditions which were the factors in determining their origin could not have produced the multi-micellar aggregates constituting the protoplasm or cytoplasm of a cell of the kind visible to-day under the microscope. As time passed, however, these micellar organisms must have gradually become multi-micellar and simple, very undifferentiated cellular forms were developed from which, later, others evolved in which structure and chemical constitution were still less simple and approximated in complexity to that characterizing the very simplest living organisms of today. These had as yet no nuclei and an enclosing membrane could have been only of the most elementary character.

From the first there must have been an adjustment in the composition of these very simple organisms to that of their medium, the ocean water, which diffusing into each minute multi-micellar mass brought into it the inorganic elements in the proportions in which they obtained in the external medium. In consequence, the internal medium was but ocean water and all the processes of metabolism, synthetic, assimilative and oxidative, were based on the physico-chemical conditions thus obtaining in the internal medium. With changes in the concentration and the proportions of the inorganic elements in the ocean water, which changes developed more slowly, the adjustment between the external and internal medium continued, for until the organisms developed membranes diffusion into their cytoplasm was unaffected and the osmotic pressure of the internal medium increased gradually and equally with that of the external medium, while the assimilative and metabolic processes altered slowly and correspondingly.

These organisms synthesized their own constituents from the available nitrogen and carbon dioxide of the air, the sulphur of the sulphates, the phosphorus of the phosphates, and the iron in the sea water, just as a number of plankton organisms do today. The proteins and other products resulting and constituting the living complex, were in special association with the sodium, potassium, calcium and magnesium of the internal medium and constantly tended to develop fixed relations with these elements.

There was as yet no nucleus. The cell must have divided very often irregularly and unequally, and although there were acquired characters, these were not necessarily transmitted in the division to both daughter cells. Iron-holding nucleo-proteins (chromatins) were undoubtedly present which controlled the transmission of acquired characters, that is heredity, but the certainty of their function must have been affected by the inorganic elements, for, otherwise, a nucleus would not have developed.

The contents of the normal cell nucleus, as the author has found, does not know the inorganic world. It contains not a trace of potassium, or of chlorides, phosphates, carbonates or sulphates, and accordingly, no sodium, calcium or magnesium, although these four elements are found in the cytoplasm. As this is true of the nucleus of the vegetable as of the animal cell, it must have developed before the differentiation into the two types began, just as did also mitosis, the phenomena of which are still so similar in both. The absence of inorganic elements from the nucleus suggests that the latter evolved to serve as a sanctuary

to protect the chromatin, the heredity-controlling factor, from being in the slightest degree affected by the inorganic salts in the cytoplasm.

The development of a cell membrane that would limit the diffusion into the cytoplasm of the salts in the external medium would more or less limit the concentration of the sodium, potassium, calcium and magnesium already there and in certain relative proportions as well as concentrations, to which because of the previously long action on the cells the cytoplasm had become adjusted and which, accordingly, became more or less fixed. There would thus tend to establish relations which would be perpetuated and the result would be an endowment on the inorganic side, evidence of which one would, on first consideration, expect to find in the higher Metazoa, the cells of which have been bathed for many millions of years with a plasma constant in its inorganic composition.

How much this expectation is realizable, or can be realized, may now be discussed.

For such evidence we may turn to the results of analyses of the inorganic constituents of the tissues of Vertebrates, and especially those of the various organs of the same, which are on record. A considerable number of such analyses have been made, but as Katz (1986) points out for those made on muscle before his time, the methods used in making them were faulty and the results, those of Katz excepted, are very much open to question. Even if the methods were such as to give correct results, there would still be the objection that from the tissues used in making these analyses, those of Katz for muscle also, no efforts were made to remove the blood and lymph. Further, as in the case of muscle, the connective tissues and blood vessels were necessarily included and in these it cannot be postulated that the inorganic composition is the same as that of striated muscle fiber alone. Indeed it is almost impossible to get a mass of muscle fibres free from adventitious tissue, for although the visible blood vessels, nerves and fat may be easily removed, the finer elements and connective tissues remain and constitute a not inconsiderable portion of the same.

If one could obtain a mass of striated muscle fibres alone the results of analyses of it would not be representative of what one might find in less highly differentiated structures, for the striated muscle fibre is highly specialized, its proteins differ from those in the cells of other organs, and moreover, there is in the sarcous elements of the fibres a variety of hemoglobin which is intimately associated or combined with all the potassium they contain. The figure, therefore, for the potas-

sium content of striated muscle fibre would not necessarily be representative, any more than that for the potassium content of a red corpuscle would be of the potassium content in unspecialized or undifferentiated animal cells.

How unrepresentative of the fundamental proportions of the inorganic elements in undifferentiated cells must be the results of the analyses of muscle tissue, including its adventitious structures, may be seen from inspection of the ratios calculated from Katz's determinations:

	Na	K	Ca	Mg
Man	100	400	9.3	26.4
Dog	100	354	7.26	25.1
Rabbit	100	870	40.0	60.5
Pike	100	1415	145.0	105.0

Exceptions may be taken also to accepting the results of the analyses of the organs as representing the inorganic composition of undifferentiated cells. The liver cells, which constitute only a part of the organ and which secrete into the bile sodium and potassium glycocholate and taurocholate, and the renal tissue which is engaged in removing sodium and potassium salts from the blood plasma, therefore, cannot be used in determining the fundamental inorganic composition of the cell. How varying the results which analyses of them would give is indicated in the determinations of the potassium in each of the organs of the dog made by Olmer, Payan and Berthier (1923). They found it to range from 0.27 per cent in the suprarenals to 0.5 per cent in the muscles.

One must then turn to cellular elements of the as yet undifferentiated kind to serve for analyses of the inorganic composition in order to ascertain what are the basal concentrations for sodium, potassium, calcium and magnesium which cannot be variable and which may be of a fundamental origin or paleogenetic. Such cellular elements would be Protists of a fresh water or even of a marine habitat. Unfortunately, however, it is not possible to get these together in sufficient number to furnish enough material for analysis. Equally serviceable would be leucocytes, but the same difficulty is encountered as in the case of the Protists.

There are also such undifferentiated cells in unfertilized minute ova of Vertebrates, and especially of fishes and amphibia. In each of these

there is but one cell and with a quantity of yolk and inorganic salts in reserve for the cells which after fertilization, and as the ovum develops are derived by division from the primary cell. The concentration of the inorganic elements in such an unfertilized ovum would then be, after development begins and before the cells are differentiated to any degree, representative of the concentration in each of them.

A large quantity of the ova of the herring, *Clupea harengus*, all unfertilized, collected by Prof. J. B. Collip at the Atlantic Biological Station of Canada in 1914 for the author has been analyzed by the latter. The ova were in the completely desiccated condition which in weight represented on the average 30 per cent of that of the fresh ova. The results of the analyses are, in per cent of the total solids:

Na	K	Ca	Mg	Cl
0.275	0.59614	0.0315	0.0515	0.9813

These converted into the values for the original ova would be:

Na	K	Ca	Mg	Cl
0.0825	0.1788	0.00945	0.01545	0.2944

The ratios of these elements on the basis of Na = 100 are:

Na	K	Ca	Mg	Cl
100	216.7	11.4	18.7	356.8

These are in marked contrast with the ratios found in the blood plasma of Vertebrates. They are also, so far as calcium and magnesium are concerned, in contrast with those postulated, in the preceding section, as obtaining in the ocean water of the Archean at or before the time when life appeared in it.

As 98.6 per cent of the chlorine found must have been combined with the sodium and potassium present there would remain only 1.4 per cent to combine with the calcium and magnesium determined, and as, further, no phosphates or sulphates were found in acid extracts of the dried ova, it was suggested that a part of the calcium and magnesium found in the ash must have been in the ova, not as inorganic salts, but in combination with organic compounds, and accordingly an at-

tempt was made to determine how much of these two elements was present in the water-soluble form, that is, as inorganic salts.

Weighed quantities of the desiccated ova were placed in a Soxhlet extraction apparatus and extracted for hours. The extraction fluids, concentrated to small volumes, were then analyzed for the four elements. The results, in per cent, as calculated for the fresh ova, were:

Na	K	Ca	Mg	Cl
0.08175	0.1795	0.00458	0.00138	0.2937

from which the ratios derived are:

Na	K	Ca	Mg	Cl
100	219.9	5.6	1.6	359.2

These ratios very closely approximate those which the author has postulated as Archean, and they may, consequently, be regarded, tentatively, as of paleochemical origin. As such they would indicate the concentration of salts and ratios in the simple unicellular animal organism when or before the time the cell nucleus was evolved to protect the chromatin from being affected by the inorganic elements in the cytoplasm derived from the ever-increasing content of these in the external medium, the ocean water.

As to the time in the history of the ocean to which these ratios date back one may attempt, also tentatively, to determine. This cannot be done by comparing the total concentration of the salts (0.5905 per cent) in the herring ova with that of the salts (3.5 per cent) in the ocean water of today, for the relative proportions of the inorganic elements in the ocean have greatly altered from the first in the latter, the potassium having constantly decreased, and this introduces a confusing factor. It is, however, possible to avoid any considerable error by using as divisor, the concentration of the sodium in the ova, and as dividend that of the sodium in the ocean water, in which from the first it has ever steadily increased, and obtain an estimate of the proportion of geological time which had passed when the paleochemical ratios in the cytoplasm became fixed. As the sodium in the ova amounts to 0.08179 per cent, and that in the ocean is 1.0 per cent, it may then be inferred that one-twelfth of all geological time had intervened between the beginning of oceanic history and the fixation of the inorganic con-

centration and ratios in the cytoplasm. Further, as the amount of sodium in the blood plasma of the higher Vetebrates ranges around 0.3 per cent, it may be estimated that inorganic concentrations in the cytoplasm of undifferentiated animal cells became fixed after about one-fourth of the time passed which intervened between the formation of the oceans and the appearance of Eovertebrates.

These estimates are offered with some reserve, as analyses of the inorganic composition of other simple, undifferentiated cellular elements, which will certainly be made, may not give exactly the same results, and thus corrections may then be shown to be necessary.

Apart from all these estimates, however, it is certain that there is a chemical endowment of an inorganic character in the cytoplasm of undifferentiated animal cells, an endowment the origin of which may be regarded as more ancient than that of the inorganic chemical endowments in blood plasma of Vertebrates. It may be held, of course, that this endowment is not necessarily of very remote origin, and that the concentrations and the ratios of the various elements, sodium, potassium, calcium and magnesium are in the cytoplasm to meet the needs of the latter in its activities. It may, however, be maintained that these proportions and ratios are the result of a gradual adjustment, brought about in the far past, between the cytoplasm and its internal inorganic content, an adjustment that became stabilized when the superficial layer of the cytoplasm acquired the power of regulating the diffusion of the inorganic salts into or from it. Were it not for this regulating function there would be a correspondence in inorganic composition between the cell and its external medium. The difference between them is great and especially as regards potassium, for in the blood plasma of Vertebrates its concentration ranges in the different forms, as shown above, between 0.015 and 0.027 per cent, while in herring ova it is 0.1788 per cent, which constitutes a ratio, on the average, of 1 to 8. This is in contrast with the ratio for sodium in the plasma to that in the ova, which is 4–5 to 1.

This regulating function is not more extraordinary than is that of the membrane of the nucleus which in the vegetable as in the animal cell contains no inorganic salts, although these are present always in the cytoplasm. This function of the nuclear membrane must have originated when the cell nucleus developed, in the Archean, to prevent, as already explained, the chromatin from being affected by the inorganic salts of the cytoplasm. Why, therefore, may we not regard the concentrations and ratios of the inorganic elements in the cytoplasm,

whatever these may, on further analyses, be definitely ascertained to be, as of an origin as remote in geological time as that of the cell nucleus?

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THE PHYSIOLOGY OF PLAIN MUSCLE

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Comparison of the general summaries of the physiology of plain muscle which have been written in the past (Mayer (182), Nasse (208), Grützner (118), or du Bois Reymond (22)), with the existing state of knowledge shows that in spite of the publication of hundreds of papers our information is still very indefinite. There are two good reasons for this. First, the fact that general conclusions respecting the properties of plain muscle have often been wrongly drawn from experiments on one type of muscle in one species of animal; secondly, the fact that we have not in plain muscle such a simple, or apparently simple, relation of the tissue to its nerve supply as we have with skeletal muscle; for all, or nearly all plain muscle receives a double nerve supply, of approximately opposite functions, and we have no method by which we can eliminate the influence of the nerve supply as we can in skeletal muscle by the application of curari. Calling the nerve supplies of a particular portion of plain muscle A and B respectively, the effect of any particular treatment may be upon either *a*, the muscle cells; *b*, the nerves A or their endings; *c*, the nerves B or their endings; *d*, ganglion cells connected with A; *e*, ganglion cells connected with B, or with combined effects of one sort or another, and it is often difficult or impossible to distinguish between these.

Structure. There is a good deal of diversity of opinion as to the detailed structure of the cells of plain muscle. In the living state, according to Lewis and Lewis (167), the cytoplasm shows no fibrillation, though it contains numerous thread-like mitochondria, some fat globules, and a few granules vitally stainable with neutral red. Many authors, however (239, 257), consider that the cytoplasm contains longitudinally running fibrillae, which show double refraction, the superficial fibrils (boundary fibrils of Heidenhain) being coarser than those more centrally placed. Individual fibrils, each less than 1μ in thickness can certainly be teased out from cells treated in special ways, such as fixation in nitric acid, followed by washing in water and treatment with osmic and acetic acid. These fibrils stain with eosin. Even if

the fibres are to be considered as artefacts, their formation is of some significance perhaps. The nucleus, and the centriole, sometimes double, which lies near to it, are imbedded in a little granular undifferentiated cytoplasm, which occasionally contains some pigment (284).

At the surface of the cell there is a delicate homogeneous layer (Tiegs, 257), sometimes regarded as a mere surface condensation of the cytoplasm; it is much less definite than the sarcolemma of striated muscle, but has been seen to wrinkle during contraction (167). In many cases, if not in all, each cell is connected with its neighbours by fine protoplasmic intercellular bridges (14, 41, 274), and unless we are to regard these as artefacts (as Tiegs does, 257), we must believe that the tissue forms a syncytium, which the embryological investigations of Miss McGill (174) renders quite likely.

Roskin (230) has recently studied the comparative histology of plain muscle in several species of animals, and found that in their general arrangement these all conform to a common type. According to him the cytoplasm contains three structural elements: *a*, the envelope to which are connected the fibrils; these are of elastic nature, not contractile, and have a skeletal function; *b*, the outer layer of fluid kinoplasm, which is the contractile material; *c*, a centrally placed mass of sarcoplasm, in which is placed the nucleus, and which is the trophic portion of the cell. The fibrils are especially abundant at the surface of the cell.

There are, he believes, indications of a delicate membrane separating the sarcoplasm from the kinoplasm. Roskin considers that it is a mistake to regard plain muscle as a simplification of striated muscle.

Microchemical investigations are scanty; glycogen has been reported in the cells of plain muscle in arteries, and in the iris and intestinal muscle (158, 222): the presence of potassium at the surface of the cells has also been indicated (171).

It is extremely likely that there is more than one kind of plain muscle. At all events, this is so developmentally, for sometimes, as in the sphincter and dilator pupillae muscles, or the muscle of the sweat glands, it is developed from the ectoderm, though more usually it arises in the mesoderm layers. Lewis and Lewis (167) have followed the formation of plain muscle cells from the mesenchyme cells of the chick amnion, and find that it appears about the 4th to 5th day. The cells become spindle-shaped, and the cytoplasm when fixed shows the highly refractile, anisotropous material which seems to be inseparable from contractile substance wherever it occurs. Miss McGill (174) finds that the muscle of the alimentary canal of the pig is developed from a syncytium

of mesenchyme cells which surrounds the entoderm, and in her opinion the tissue remains a syncytium throughout life.

Nerve supply. There is usually, if not invariably, a double nerve supply, the presence and nature of which renders the study of the physiology of plain muscle distinctly difficult. The nerve fibres are generally non-medullated, and often exhibit on their course varicosities which are commonly regarded as ganglion cells, e.g., in the plexuses of Auerbach and Meissner in the alimentary canal.[1] But many of the cells in close relation to plain muscle cells are now believed to be not nerve cells, but connective tissue cells. The terminal nerve fibrils form a fine plexus on the surface of the muscle cells, and terminate, some say, in small knobs, one to each cell. According to Boecke, and Tiegs (257) the terminal nerve fibrils in the ciliary muscle actually enter the substance of the cell, to terminate there in loop-like expansions, which Tiegs believes end near to, or upon the nucleus. The nerve endings in the retractor penis of the rat and hedgehog were studied by Fletcher, (95) who found no evidence of these terminal knobs or of the tree-like branchings of other investigators, but merely a fine intercellular network stainable with methylene blue. Both the motor (sympathetic) and inhibitory (pelvic visceral) nerves took part in the formation of this plexus, and the two kinds of fibre could not be separately recognised. Though section of both nerve supplies was followed by degeneration of the entire nerve plexus, the section of either supply alone was not followed by any appreciable change in the richness or arrangement of the nerve net.[2] No nerve cells were found either by Fletcher, or subsequently by Fisher. Similar histological arrangements were found by Hofman (139) and by Tiegs (l. c.) but the latter thinks each nerve supply remains separate.[3]

The nerve supply to plain muscle is in some cases perhaps peculiar inasmuch as each of the two nerve supplies may contribute to the

[1] Elliott (1906) suggests "that the nucleate protoplasmic mass of the motor nerve ending in striated muscle, and the visceral ganglion cells of the nerves to plain muscle are homologous and further that the visceral ganglion cells in vertebrates are not derived from the central nervous system, but are of peripheral origin."

[2] In recent studies S. E. Johnson (154) finds that when the nerves to the small intestine are divided, the nerve fibres between the cells of the myenteric plexus disappear, but the cell processes to the muscle cells remain.

[3] Investigations by Miss C. J. Hill have also shown that in Auerbach's plexus the ganglion cells are connected synaptically with the vagus, while the sympathetic fibres remain distinct.

formation of the terminal structures and be competent to save this structure from degeneration when the other source of innervation is lacking. It is difficult to reconcile this fact with the admittedly distinct actions on plain muscle of the two nerve supplies when these are separately stimulated, but on the other hand it may throw some light on a certain overlapping of function which is demonstrable in some instances. As examples of this we may consider the effects of stimulation of the vagus on the stomach, which have been recently studied by M'Crea, M'Swiney and Stopford (172), and by Veach (264). The former investigators find that when the stomach is in a relaxed condition, vagal stimulation causes contraction, while when it is contracted the result is relaxation. Similar results have been obtained by other investigators, including Veach, but the explanation which is advanced by the latter is that the inhibitory effects are the results of a phenomenon allied to the Wedensky inhibition. The relations of the nerves to plain muscle is further rendered obscure by the fact that in some individuals there is voluntary control, e.g., of erection of the hairs (Maxwell, 183).

A peculiar feature of the double innervation is seen in the augmented sensitiveness of muscle which has been partially denervated. The best known example is the pupil after removal of the superior cervical ganglion, which becomes much more sensitive to adrenaline: according to Meltzer and Auer (199), and Shimizu (243), the same is true of the rabbit's ear vessels, and Elliott (84) finds that it also holds for the dog's retractor penis, the cat's iris and the cat's bladder when completely denervated. Shimidzu (l. c.) also claims that it holds good for the completely denervated intestine. Since there seems good evidence that the exaggerated response to adrenalin is obtained with the plain muscle deprived of both sources of innervation, it does not seem possible to explain it on the lines of the persistence of the peripheral nerve network after partial denervation as claimed by Fletcher; the most likely explanation seems to be on the lines suggested by Elliott; namely, that it is connected with the persistence of receptor substance with enhanced sensitiveness.

Relation of structure to function. According to Carey (53), the structure of muscle is determined by the amount of tension to which its fibres are subjected. He claims that if the pressure within the urinary bladder is raised for 8 weeks to the same level as that in the heart cavities, there is a transformation of the plain muscle fibres into transversely striated and rhythmically contractile fibres. The physiological differences between different varieties of muscle according to this view is their

capacity for work, and it is concluded that it is function which determines structure, and not the reverse. This view cannot be accepted without further evidence. It has long been known that at places where there is a change from striated to plain muscle, e.g., in the oesophagus (114), the change is quite sudden, there being no transition form between the two. Again, the ciliary muscle of birds is of striated muscle fibres, yet there is no clear indication that its function in substantially different from that of the corresponding muscle in mammals. The regeneration of smooth muscle has been studied by Berry (19).

The tonic properties of the tortoise auricle (33) are probably connected with its presence of a layer of plain muscle beneath the endocardium (242). (Elastic fibres in plain muscle, see (287).)

Histological changes in functional activity. When sections of plain muscle which have been fixed in the contracted and relaxed states are compared, it is found that these exhibit no such striking differences as are seen with striated muscle under similar conditions. The chief difference seems to be that there is a diminution in volume of the cells when they contract, since the interstitial spaces are more pronounced, while the cells themselves appear of a denser consistency, and stain more deeply. The nuclei are also thicker and shorter, but the changes which they undergo are no doubt purely passive (180). Schultz (239) claims that these observations are incorrect, and that the cells become thicker on contraction, but Meigs (191) has upheld the view that the cells exhibit shrinkage, when caused to contract by immersion in Ringer's solution which has been made alkaline, or to which barium chloride has been added. That the contraction is associated with an increase in the density of the cytoplasm is indicated by the increased depth of staining: fixed preparations often show nodes of thickening staining with iron hematoxylin, at one or more places in the cell, and these are believed to be sites of contraction. Also, in cross section, some of the cells are more deeply stained than others and Heidenrich (130) and others consider the dark-stained cells to be those fixed in a state of contraction. The contracted nodes according to Soli (cit. Roskin, 1923) show strong double refraction. Tiegs, however, appears to regard them as a kind of primitive cross-striation (257).

The histological changes in contraction of the living muscle were studied by Lewis and Lewis (167) in the chick amnion cells in tissue culture. They found that when a spontaneous contraction took place, there was a flowing of material from fixed points towards a central point, which thereby became thickened, while the portions between this

and the fixed points were thinned, and sometimes actually broke across. Movements of structural component of the cell, such as nucleus, mitochondria, etc., were purely passive. Essentially similar flowing movements of the kinoplasm of the cell contents into expanded nodes have been described by Roskin (230). The latter also finds that the contracted portion loses its transparency and becomes milky so long as the contraction lasts; as soon as relaxation occurs it becomes clear again.

A curious phenomenon connected with the extension of plain muscle has been described by Grützner (118). If the stomach of a fasting frog be examined in section, it is found to have a muscular layer composed of about 20 layers of cells, whereas the distended stomach of a well-fed frog of the same size shows, at the same part, only about 4 or 5 layers. If this observation be accepted as correct, it follows that the enlargement of the organ when it is filled is due not only to stretching of the individual fibres, but also to a considerable extent to movement of these one over the other (283). An objection to this view of displacement is, however, that definite cell bridges are believed to occur which connect each cell with its immediate neighbours. It should be mentioned in this connection that Mr. H. P. Gilding has recently investigated this suggestion of Grützner's in my laboratory, and has found little evidence to support it.

In plain muscle another way in which the fibre gives way under extension is by alteration of its length without its exerting any increased tension; in this respect it behaves like a rod of very viscid material, such as half-melted glue. Anyone who has attempted to mince plain muscle must have been struck by the great viscidity of the material. When plain muscle is slowly and moderately extended, therefore, its length is increased, chiefly by passive stretching of the viscous substance, slightly perhaps by displacement of cells, and scarcely at all by mere elastic stretching with increase of tension.

Chemical composition of plain muscle. For the study of the chemical composition of plain muscle, materials from very different sources have been used, e.g., the retractor penis (63, 66, 89), fibromyomata (138), stomach (89, 232) and intestine (89, 206), bladder (89, 232, 240), uterus (63, 66, 89), various muscles of invertebrates (150, 189, 277), etc. The results naturally exhibit considerable variations, according to the source of the tissue employed, but certain common facts emerge, and it is only to these that any detailed reference need be made here.

The water content is about 80 per cent (63, 138, 232), i.e., somewhat more than in striated muscle, the total solids about 20 per cent, of which

1 to 3.5 per cent is ether soluble (232), about 14 to 18 per cent protein, 0.8 per cent ash, and traces of various other substances. Watery extracts are alkaline or neutral, but the press-juice (24) is sometimes faintly acid.

Proteins. In striated muscle, about three-quarters of the total protein is myosinogen, the remainder being para-myosinogen. In plain muscle, both of these proteins are present, (118, 263, 271, 272) the chief one found in saline extracts being myosinogen (272). The saline extracts, or press juice show spontaneous coagulation on standing. Saxl (236) finds that the soluble ("plasma") proteins form seven-eighths of the total protein in striated, one-third in cardiac, and one-quarter in plain muscle, and of this soluble protein one-fifth is paramyosinogen and four-fifths myosinogen. Saiki's findings are similar. It is claimed that if we accept the view that rigor mortis is associated with coagulation of soluble proteins, we have in this an explanation of the fact that rigor mortis is seen most clearly in striated muscle, and not in plain muscle. The isoelectric point of myosinogen is pH 6.3, while that of paramyosinogen is pH 5.15 according to Weber (273), which perhaps accounts for the fact (272) that more paramyosinogen was found in extracts made with acidified salt solutions than in those made with water. Hemoglobin is also present in the tissue in certain situations, e.g., the gullet of the snail and the human rectum (163).

A striking difference in the proteins as compared with striated muscle is in the amount of nucleoprotein, which is much greater in plain muscle (26, 263, 272) (about ten times as much). Hence a considerable amount of the phosphorus present is in the form of nucleoprotein.

The distribution of the nitrogen of the proteins has been studied by Buglia and Costantino (46), who find that the proportion of ammonia, mono- and di-amino nitrogen is much the same as in striated muscle, i.e., it is chiefly in the form of mono-amino nitrogen (277), though in extracts of the dried coagulated tissue, di-amino nitrogen preponderates. The protein sulphur is also similar in amount to that in the protein of striated muscle (65).

Creatine and creatinine. Numerous investigations (16, 46, 50, 96, 206, 224, 232, 240, 241) have shown that both substances are present, though in much smaller amount than in striated muscle. According to Mellanby (198) and Wright Wilson (277), creatine is absent from invertebrate muscle, but is found in vertebrates from the lamprey upwards. The results of some of these estimations are given in the following table.

TABLE 1

Creatine content of plain and striated muscle

ANIMAL	MUSCLE		PER CENT (AS CREATININE)	OBSERVER
Rabbit	Mixed		0.450	
Rabbit	White		0.454	Riesser (224)
Rabbit	Red		0.254	
Ox	Striated		0.403	Beker (16)
Dog			0.387	Folin and Buckman, l.c.
Pig			0.338	Beker
Human			0.355	Myers and Fine (206)
Cat	Extensor l. digitorum.		0.427	Scott and Spohn (240)
Cat	Soleus		0.278	
Dog	Cardiac		0.249	Folin and Buckman
Rabbit	Large Intestine	Plain	0.0325	Beker
Rabbit	Small intestine		0.0234	Beker
Ox	Uterus		0.038	Beker
Ox	Bladder		0.094	Mario Cabella (50)
Dog	Retractor penis		0.097	Buglia and Costantino (46)
Pig	Uterus		0.03	Beker
Pig	Stomach		0.086	Saiki (232)
Pig	Bladder		0.078	Saiki (232)
Human	Uterus		0.077	Riesser, l.c.
Cat	Bladder		0.097	Scott and Spohn

Creatinine contents

ANIMAL	MUSCLE	PER CENT	OBSERVER
Dog	Striated	0.0047–0.0108	Shaffer (241)
Ox	Striated	0.0118	Shaffer
Human	Striated	0.00498 (mean)	Myers and Fine
Human	Striated	0.01 (mean)	Shaffer (241)
Human	Uterus	0 0012–0.0022	Myers and Fine
Cat	Extensor. l. digitorum	0.0048	Scott and Spohn
Cat	Soleus	0.0042	
Cat	Bladder	0.01	

These results indicate that creatine and creatinine are present in plain muscle in about one-tenth the amount present in striated muscle.

Other extractives. Carnosine has been found by Buglia and Costantino

(47), but in smaller amount than in striated muscle. Histidine (or carnosine ?) was also found by Jansen (150) in Mytilus. He also found betaine, as also did Wright Wilson (275, 276), in invertebrate plain muscle. Taurine was identified by Jansen (l.c.)

Purine bodies are stated by Buglia and Costantino (47) to be present in smaller amount than in striated muscle, although the nucleo-protein content is higher; unlike striated muscle the principal purine base is according to them not hypoxanthine but xanthine. Saiki finds that hypoxanthine is the chief purine body, and also found adenine and guanine.

Choline has been isolated by Le Heux (134), from extracts of small intestine, made by laying the peritoneal surface in contact with water; the substance has been isolated and recognised by the melting points of the mercury, gold and platinum salts, and by various microchemical tests. It is not known whether this substance is present in other forms of plain muscle. Girndt (111) finds that the intestinal choline is not derived from the suprarenals, and that it cannot be formed anew in the isolated intestine. Geiger and Loewi (108) find an increase in the choline content of frog's striated muscle as a result of stimulation, and are of the opinion that it is formed from lecithin.

Fats and lipoids. The amount of substances soluble in light petroleum is about the same as in striated muscle, and less than in cardiac muscle (Costantino, 64, 66, 67). It appears that in plain muscle most of the higher fatty acids are present as phosphatids soluble in 95 per cent alcohol, and four-fifths of the higher fatty acids are present in the lecithin fraction. Of the alcohol-soluble fraction three-fourths of that soluble in ether and acetone consists of unsaponifiable matter. During autolysis it is stated (66) that the soluble and inorganic phosphorus is increased, being apparently liberated from protein compounds by the action of autolytic enzymes.

Carbohydrates. The carbohydrate of principal interest is of course glycogen, but although histologically demonstrable (158, 222) this is present only in small amount in the plain muscle of vertebrate animals (232). In the adductors of Mytilus, Jansen (150) found as much as 1.5 per cent of glycogen, and also claimed to have isolated a crystalline pentatomic alcohol of the empirical formula $C_6H_{12}O_5$ to which he gave the name *mytilit.*

I have carried out estimations of glycogen in the bullock's retractor penis, and in the stomach of the cat and of the tortoise, by the usual Pflüger's method, and at first found that the amount was apparently

of the order of 0.02 per cent to 0.05 per cent only. It was subsequently discovered, however, (89) that these amounts were too low by several hundred per cent. The explanation was that in the use of the relatively large volumes of precipitating alcohols, there was considerable loss of glycogen when the amount present was small. As a result of comparative estimations of glycogen on varying small quantities of striated muscle, it was found that the volume of fluids from which the glycogen is precipitated should not exceed 1 cc. per milligram of glycogen.

The method which I now use for the estimation of glycogen in plain muscle is as follows: The tissue (5 grams) is placed in 30 cc. of 75 per cent alcohol cooled down to −5°C. The tissue is minced finely with scissors, while in the cold alcohol, and is then transferred to the refrigerator until next day. The alcohol is then poured off and filtered, the residue wrung out in muslin, and extracted with 5 cc. of 75 per cent alcohol for an hour. This is also filtered off, and the extraction repeated with 75 per cent alcohol twice. The filtrates are combined, and may be used for the determination of soluble carbohydrate and lactic acid.

To the residue a few drops of saturated sodium bicarbonate solution are added, and it is then placed over sulphuric acid in a vacuum desiccator overnight. The dry residue is transferred to a centrifuge tube, and 1.3 cc. of 30 per cent potash is added; the mouth of the tube is closed with a glass bulb, and it is immersed in a boiling water-bath for several hours. Any insoluble residue removed by centrifugation is thrice washed with 1 cc. of 15 per cent KOH. To the combined potash fluids 6.5 cc. absolute alcohol are added, and the contents centrifuged. After pouring away the supernatant fluid, the residue is stirred up with 0.5 cc. of 15 per cent potash, then 1 cc. alcohol is added, and the deposit again centrifuged: the residue is taken up in 0.5 cc. of 0.1 per cent NaCl, and thrown down by 1 cc. alcohol. Finally the residue is mixed with 5 cc. of 2.2 per cent HCl, the mouth of the tube loosely stoppered with a glass bulb, and the contents heated for 3 hours in a boiling water bath. The hydrolysate is made to a definite volume (10 to 25 cc.) and the glucose content estimated by any suitable method. One mgm. glucose = 0.927 mgm. glycogen. (If the method is to be used for striated muscle, the amount of tissue may be as little as 0.3 gram. For plain muscle, from 5 to 10 grams are necessary.)

By the use of this method it was found that the glycogen content of the tortoise stomach was about 0.15 per cent, which, as will be shown later, is a fact of considerable importance.

Uncertainty prevails concerning the other carbohydrates of plain muscle. By extraction with alcohol (89), a reducing substance equivalent to about 0.15 to 0.05 per cent of glucose is obtained: the fresh press juice of fibromyomata was found by Hirsch (138) to contain about 0.85 mgm. per cubic centimeter, but it fell off to about one-fourth of that amount in 24 hours. Estimations of the total carbohydrate of the tissue by hydrolysing the fresh muscle with 2 per cent HCl, followed by

deproteinisation by mercury, gave results much higher than the sum of those obtained by separate determination of glycogen and of alcohol-soluble carbohydrate. Thus in the tortoise stomach tissue, the total hydrolysable carbohydrate is of the order of 0.5 per cent, or about twice as much as the sum of glycogen and alcohol-soluble carbohydrate. It seems certain then, that some reducing substance is generated in the tissue in the course of the hydrolysis: It is conceivable that this may be from nucleoprotein, but I have not investigated this point. At all events, I do not think that the carbohydrate metabolism of plain muscle can satisfactorily be investigated by the determination of total reducing substance formed on acid hydrolysis; Meyerhof, too, has found that in striated muscle, the hydrolysable carbohydrate is rather too high.

Lactic acid. Sarcolactic acid has been identified by Meigs (184) and by Saiki (232), who found about 0.05 per cent in the stomach and 0.07 per cent in the baldder muscle of the pig. Large amounts of tissue were required for the separation, which was by means of the zinc salt. I have also separated the zinc salt of lactic acid from the plain muscle of the horse stomach, the bullock retractor, and the dog's intestine, and found quantities of the same order. More modern methods of estimation have allowed me (89) to estimate the lactic acid in much smaller amounts of tissue and to study the conditions of its formation. The amount is usually lower than would be expected from comparison with striated muscle, though in no case was lactic acid found to be absent. The resting values are not comparable with those of striated muscle, and are higher than the minimal value found for that tissue.

Salts. Analyses of the ash of plain muscle have been made by Saiki (232), Meigs and Ryan (189), and Costantino (63). Some of the results are given in the following table.

Consideration of this table indicates that Saiki's analyses differ widely from the remainder, and cannot readily be accepted. It shows that the content of potassium and of phosphorus is a little less than in skeletal muscle, while that of sodium like that of chlorine, is definitely greater. This means that there is more sodium chloride and less potassium phosphate in plain muscle than in striated, particularly when we remember that some of the phosphate of plain muscle is derived from the nucleoproteins during the incineration. The ratio of sodium to potassium (1:1.5) is very different from that in striated muscle (1:5). In plain muscle, the sum of the Cl and PO_4 ions is stated to be less than the sum of K and Na, whereas in striated muscle the reverse holds good (64).

The phosphorus is present, partly as inorganic phosphate, but also in part in organic combination, e.g., as phosphatids and nucleoproteins. Hence the distribution of phosphorus is very different from that in striated muscle.

TABLE 2

Plain and striated muscle, water and inorganic elements per 100 grams

SOLIDS	WATER	K	Na	Fe	Ca	Mg	Cl	S.	P.	TISSUE	OBSERVER
19.4	80.6	0.067	0.243	0.008	0.032	0.004	0.171	0.087	0.081	Pig stomach	Saiki (232)
17.7	82.3	0.325	0.073	0.0007	0.004	0.013	0.120	0.161	0.137	Frog stomach	Meigs and Ryan (189)
20.2	79.8	0.366	0.089				0.105			Ox stomach	Costantino (63) (examples selected at random)
20.4	79.6	0.267	0.109				0.128			Ox retractor	
18.3	81.7	0.225	0.154				0.111			Cow uterus	
21.2	78.8	0.356	0.072				0.083			Fowl stomach	
20.1	79.9	0.350	0.054	0.01	0.028	0.030	0.066	0.141	0.155	Frog striated	Meigs and Ryan (189)
27.1	72.9	0.254	0.156	0.006	0.008	0.028	0.048	0.204	0.213	Striated	Katz (cit. Saiki)
25.2	74.8	0.325	0.049				0.028			Dog striated	Costantino (63)

TABLE 3

After Costantino (64)

	DISTRIBUTION OF PHOSPHORUS IN PERCENTAGES IN					
	Striated muscle	Plain muscle of				
		Retractor		Stomach	Uterus	
	per cent	*per cent*	*per cent*		*per cent*	*per cent*
Inorganic P	81.5	40.8	48.0	52.4	39.7	29.2
Organic P	18.5	59.2	52.0	47.6	60.3	70.8
Phosphatid P	16.4		24.0	25.2	19.5	28.4
Other organic P	2.1		28.0	21.4	40.9	42.4

The table given below (from Costantino) illustrates this difference. In striated muscle it will be seen that nearly all the organic phosphorus is that present as phosphatids, while in plain muscle only half or one-

third of the organic phosphorus is present in that form. The table shows that in plain muscle the inorganic phosphorus is definitely lower than in striated muscle.

The buffering power of the inorganic materials in plain muscle is probably low when compared with striated muscle; some substances capable of fixing carbon dioxide are present however, for Hirsch (138) finds that the press juice of fibromyomata tissue has an alkali reserve of 35 to 50 vols. per cent.

PHYSIOLOGICAL PROPERTIES OF PLAIN MUSCLE. The physiological properties of plain muscle have been already described by Grützner (118), du Bois Reymond (22) and others, and it will only be necessary here to add a few details to the general description which they have given. Great differences are met with in the plain muscle from different sources, but viewing the question as a whole, it may be said that plain muscle is distinguished from striated muscle by the sluggishness of its contractions, by the exhibition of tonus, and by often showing spontaneous rhythmic contractions.

The contraction of plain muscle has been observed and studied in a great variety of preparations, among which may be mentioned in mammals the uterus (90, 142), ureter (147), vas deferens (112, 220), fallopian tubes (62), retractor penis (37, 82, 281), esophagus (114, 148), stomach (42, 255), small intestine (116, 119, 121, 125), large intestine (234), vermiform appendix (122, 141), urinary bladder (18, 78, 82, 251, 253), muscles of the bronchi (258), muscularis mucosae (121, 256), vagina (120), spleen (237), coats of arteries (143), and veins (36, 98), iris (54, 123, 266), arrectores pilorum (157), among other species the hen's esophagus (93), the stomach (5, 74, 75, 239), and esophagus (33) of the frog and tortoise (89), the frog's bladder (55), the crop (201), or body of the earthworm, the body of the leech (105, 153, 233, 254), the heart of the snail (85, 140), the adductor muscle of bivalves (20, 91, 214, 216, 260), and other forms of invertebrate muscle (43, 141, 156, 202). It seems very doubtful whether these tissues can be considered as comparable.

A convenient bath for the study of isolated plain muscle preparations is described by Burn and Dale (49).

Extensibility and tonus. Plain muscle is a highly extensible tissue, but its extensibility varies with the conditions which determine what is called its tonus. When a weight is applied to it, a certain amount of extension occurs almost at once, but this is followed by a long-lasting after extension, the muscle reaching its final length asymptotically, so

that it may take an hour or more before reaching an approximately constant length. This slow alteration is certainly mainly due to the high viscosity of the cytoplasm, though Grützner (118) has suggested that it may also be in part due to a sliding of the muscle cells one over the other.

The tonus of muscle may be defined as a resistance of its substance to extension. We could say that the degree of tonus is $= F/L$, where F is the stretching force and L is the final length of the muscle to which it is applied; the tonus varies considerably with the conditions. Thus a guinea pig's uterus to which a stretching force of one gram is applied may at one time have a length of 5 or 6 cm., while under different conditions it may be only 1 cm.; yet in both instances the tissue would show rhythmic contractions causing small fluctuations about these mean lengths. There is thus no constant relation between initial length and initial tension such as may be seen to hold approximately with denervated skeletal muscle.

It is impossible definitely to say whether the state of tonus in plain muscle is to be regarded as of the same nature as its contractions. I can see no reason why the two should be considered as fundamentally distinct. If we regard contraction as the consequence of certain chemical and physical changes in the cell, and relaxation as their reversal to a former state, there should be no difficulty in considering a state of tonus as either a continued contraction (51), or as an inhibited or partly inhibited relaxation (88), and we shall see later that it is in all probability to be regarded rather in the latter light. From such a new equilibrium position a state of further contraction or further relaxation could be reached, and it would be quite possible for a series of contractions and relaxations to be superposed on to the state of tonic equilibrium. In such a case we say that the tissue shows contractions with good or poor tonus as the case may be.

The nature of tonus. Experiments on the adductor muscles of bivalves (20, 61, 214) have indicated that during the tonic contraction, which is often very prolonged, there is no increase in the oxygen usage of the animal, and its was inferred that the maintenance of the tonic state was not associated with any increased expenditure of energy, as would have been the case had the tonus been of the nature of a tetanus. I have been able (88) fully to confirm this observation on mammalian plain muscle thrown into a tonic state by the use of suitable drugs, such as histamine. Two different methods for estimating the oxygen usage were employed, and both gave the same results, some of which are shown in table 4.

As an average of 12 experiments it was found that the oxygen usage in the relaxed state was 0.46 cc. per gram tissue per hour, while for the tonic state it was 0.39 cc. There is therefore actually distinctly less oxygen used in the tonic state than during the state of relaxation. This is probably because, owing to the thickening of the tissue on contraction, it was able to get oxygen less readily than when it was in the relaxed state. At all events, the experiment shows that there can be no definite increase of metabolism during the upkeep of tonus. Parker (213) also finds that there is no increased production of carbon dioxide by sea anemones in tonus, though *during* the onset of contraction there is an increase of about 25 per cent.

Similarly, I have been unable to find any pronounced increase in lactic acid in muscles kept in tonus for long periods.

TABLE 4

Oxygen usages of plain muscle (Evans (88))

TISSUE	STATE	OXYGEN PER GRAM PER HOUR
		cc.
Uterus	Relaxed	0.30
Uterus	Tonic	0.25
Uterus	Relaxed	0.26
Uterus	Tonic	0.23
Intestine	Relaxed	0.15
Intestine	Tonic	0.13
Uterus	Relaxed	0.29
Uterus	Tonic	0.30

If, as we believe, the tonic state is not associated with an increase in the chemical changes coupled with the act of contraction, then it would be expected that the electrical state set up during contraction would subside once the tonus was established. This appears to be what does happen (102, 103). When the beating snail's heart is caused to pass into a state of tonic contraction, as it does when carbon dioxide is administered in high concentration, the electrical changes cease, and there is a return to an isoelectric state (85).

THE EXCITABILITY AND CONTRACTION OF PLAIN MUSCLE. When compared with striated muscle, the phenomena of contraction in plain muscle are distinguished by their relative sluggishness. The latent period, period of contraction and relaxation phase are all greatly prolonged, and are moreover much more variable than those of skeletal muscle. Plain

muscle responds to a great variety of stimuli, among which are mechanical, thermal and electrical stimuli, and excitation by light and by chemical substances. Almost any alteration, provided it to be a sudden one, in the chemical or physical conditions, can excite plain muscle.

Electrical stimulation. The galvanic current is a very suitable excitant for plain muscle; both make and break excitations occur. The effect of a stimulus depends not only on its strength but also on its duration, so that a weak current applied for a longer time may be as effective as a brief stimulation by a much stronger current. This is because the chronaxie is much longer for plain than for striated muscle. Boruttau (23) gives 2 to 2.5σ for frog's stomach, 3 to 5σ for toad's stomach, while L and M. Lapicque (164) give 1.5 seconds for the chronaxie of the blood vessels of the frog's web and 11 to 15 seconds for pigment cells. The tissue thus responds much better to a slowly rising than to an abruptly rising stimulation. In fact, for this reason induced currents are frequently ineffective owing to their short duration. During the flow of a constant current it was long ago shown by Engelmann the neighbourhood of the cathode is in a state of increased excitability, while near to the anode the excitability is greatly depressed. The excitability of the tissue also shows interesting changes according to the state of its natural tonus (51, 74, 118, 281). When the muscle is in a relaxed state, it is more excitable both to faradic and to galvanic stimuli, but especially to closure of the galvanic, and, in cold-blooded animals, to opening of faradic currents. When the muscle is in a state of tonus, closure of the galvanic current causes relaxation, and opening, a weak contraction, while faradic stimuli are ineffective (118).

Mechanical stimuli. It is well known that plain muscle is extremely sensitive to many forms of mechanical stimulation; the effect is sometimes contraction, at other times relaxation (90, 112, 146). A very important effect is that of the stream of oxygen bubbles which is generally passed into the saline bath in which plain muscle preparations are suspended for investigation (90, 112). The guinea-pig uterus, which normally shows very little tonus, responds by a strong contraction when the passage of oxygen bubbles is stopped. This result is not to be attributed to lack of oxygen, because the same consequence follows the interruption of a steam of bubbles of nitrogen: resumption of the bubbling, or agitation of the liquid by stirring, or gently brushing the tissue with a camel-hair brush cause a return to the relaxed condition. It is curious that when the tissue is in a contracted state, the effect of mechanical stimulation is reversed, its cessation now being attended by relaxation.

The cat's uterus, which is normally tonic, behaved like the contracted guinea pig's uterus. Mechanical stimulation seems therefore, to cause the muscle to do just the opposite to what it was doing when the stimulus was applied; this recalls the effect of electrical stimuli on the contracted and relaxed muscle referred to above. The rise of excitability on extension is often great enough to cause the extension to act as an adequate stimulus, as it does also in cardiac muscle, and to cause contraction, frequently of a rhythmic character. In such cases, the greater the stretching force the greater the frequency of the rhythmic contractions. This may be seen in the ureter, and also very clearly in the snail's heart, which consists of plain muscle (85). In other forms of plain muscle, a sudden stretching force gives rise to a response consisting of a single contraction; this is easily shown by giving a lever attached to such a piece of tissue a slight sudden pull, when the tissue responds by a strong contraction.

Stimulus by light. Plain muscle fibres bear a relationship to pigment cells, and like them contract to certain forms of light stimuli. It has been known since 1843 that the pupil of an excised eye often for many days after death, will contract on exposure to light, and it has been shown that it is the circular muscle which so contracts; moreover, that tiny fragments of iris, which contain only 10 to 15 muscle cells with no recognisable nervous structures, also react to light by contracting (Guth, 123).

Adler (3) has shown that visible rays have no effect on normal plain muscle, though ultraviolet rays are capable of causing contraction of many forms of plain muscle. But if tissues containing plain muscle are sensitised by treatment with fluorescent substances, such as hematoporphyrin, then visible rays will cause contraction. This has been abundantly confirmed (127, 137). It was claimed by D. T. Harris (127) that visible rays opposed the action of ultraviolet light in causing contraction of the frog's stomach: this point has recently been re-investigated by L. Hill and Azuma, (137) who deny that such interference is demonstrable, either with the stomach or large intestine of the frog, or the uterus of the guinea pig. They find that ultraviolet light can antagonise the relaxing effect of adrenalin, but not the effect of emetine; also that the presence of calcium was necessary for the production of the effect of ultraviolet light.

The action of ultraviolet light is probably a direct one on the muscle fibres, since it is produced in a plexus-free preparation of the cat's intestine. The intestine of the rabbit and guinea pig is according to Adler

(3) paralysed by ultraviolet light, probably owing to an effect on the nervous structures present.

Effect of temperature. The effects of temperature (45, 75, 79, 229, 266, 267, 269, 270, 281) vary according to the suddenness with which it is altered; sudden alteration of temperature can act as a stimulus and provoke a powerful contraction in plain muscle. Gradual change of temperature has a quite different effect, which consists of relaxation with rise and a steady tonus with fall of temperature within the ordinary limits of physiological experiment. At a certain elevation of temperature, usually between 45 and 48°C for mammalian tissues, the muscle is fully relaxed, and is no longer responsive to electrical or chemical stimuli, though it is not dead (87). If such tissue is cooled down to body temperature again, it recovers its excitability more or less completely, This phenomenon is known as *heat paralysis*, and is not peculiar to plain muscle, since it is shown by other forms of excitable tissue.

At a temperature slightly higher than that at which heat paralysis occurs, the tissue is speedily killed, e.g., at about 50°C. in a few minutes, and shows no return of excitability on re-cooling to more normal temperatures. (Paramyosinogen coagulates at about 50°C. (272).) Death by heat takes place without any contraction, in fact the tissue, unlike skeletal muscle, dies in the fully relaxed condition. If the heating is continued, however, coagulation changes occur, which results in a large heat "rigor" contraction at about 60° to 63°C. (32). (Coagulation of myosinogen, 272.) Meigs (184) is of the opinion that this shortening is due rather to loss of water than to coagulative changes.

Plain muscle, though it enters into an obstinate tonic contraction when cooled, suffers very little thereby, and is found even after days at 0°C., to have retained its vitality.

Chemical stimuli. Many chemical substances act as stimuli to plain muscle, either directly, or through the intermediation of their nerves. An account of these may be found in textbooks on pharmacology, and it is not necessary to enter into details here. It is not always, or even usually, possible to say whether the action of any particular chemical substance is a direct one on the muscle, or an indirect one on its nervous structures. There is no drug which has on plain muscle an action clearly comparable with that of curari on striated muscle, in paralysing the nerve endings. Schultz (239) and others (253) supposed that atropine would do this, but the amounts used were very great, and undoubtedly affected the muscle itself. Nicotine and cocaine are also often used for this purpose (15), (74), yet it is certain that cocaine actually increases the sensitiveness of muscle to sympathetic excitation.

Drugs which act on plain muscle through their nerves are often classed as sympathicomimetic and parasympathicomimetic drugs. The most important sympathicomimetic substance is adrenaline; among the drugs with parasympathetic action may be mentioned physostigmine, pilocarpine, eserine, nicotine (in small doses), choline and its esters (e.g., acetyl choline), arecoline, etc. On the other hand, atropine and its near relations have a paralysing action on the parasympathetic nerve endings.

Of drugs which are generally said to have a stimulating action on muscle direct, are barium salts, (121, 253) pituitrin, and histamine. There is considerable doubt whether pituitrin is to be regarded as a general stimulant however (173) Histamine causes contraction of almost all plain muscle, the chief exception being the blood capillaries, and the uterus of the rat, which are relaxed. Phenols (163) and guanidine (245) are also stated to be stimulants. Nitrites have a depressant action on the tissue (30) and so has urethane (98) and chloretone (231) and alcohols (13).

Action of ions. The saline solutions which are so frequently used in experiments on surviving tissues are of importance in two respects: first, as providing a medium of the requisite osmotic pressure; secondly, as providing certain ions.

Reduction of the concentration of the Ringer's solution in which a plain muscle preparation is immersed increases the excitability of the tissue, and the extent of its contraction in response to a stimulus (70, 115); increase of tonicity has the opposite effects. That the isotonicity is not the only function of such solutions is proved by the loss of function which occurs when the saline mixture is replaced by isotonic sugar solutions (169).

As regards the action of various ions as such, it must be confessed that the subject is at present in a chaotic condition, and seems likely to remain so until we have some idea of the manner in which the different ions produce their effects. Steps in the right direction have been made by Mines (202), and more recently by Hogben (140), but their work was on invertebrate tissues, and the results are not shown to be applicable to mammalian tissues. Hogben finds, contrary to my observation (85), that the heart of Helix is affected by potassium in the same way as other plain muscle,[4] which is caused to contract (Clark (57)), though he does not find, as Clark did for mammalian tissues, that withdrawal of potas-

[4] The only explanation for this discrepancy which I can suggest is that there may be a seasonal alteration, for my experiments were carried out in the summer, and Hogben's in the winter.

sium also produced contraction. The hearts of Helix and Pecten can beat well in the absence of potassium.

Increase of calcium depresses the cardiac rhythm in Helix (85, 140), and reduction of calcium causes systolic stoppage, (140), while the heart of Pecten shows the opposite behaviour (140, 202). The crop of Aplysia or Helix behaves in a similar manner to the heart of Helix as regards K and Ca. Magnesium acts like calcium of the crop of Aplysia; Mg is essential for the beat of the heart of Pecten.

In mammalian tissues it is generally agreed that potassium causes an increase of tone, or definite contraction. Clark (57) also finds that withdrawal of K causes increase of tone. (Jannink apparently finds otherwise (151).) Miss Soref (249), following Zwaardemaker, claimed that other elements which emit β rays, as well as free radiations, have the same effects as potassium (249,) but Clark (57) finds that rubidium is twice as active as potassium, caesium is only half as active, while thorium and uranium have no action. Hogben (140) finds similar relations for the action of K, Rb and Cs on Aplysia crop. Buglia (44) again, finds that contractions arrested by lack of calcium are restarted by rubidium salts.

Calcium has in many cases been shown to produce contractions of plain muscle when added in excess (93, 162, 256) and the same applies to strontium and barium, even in small amounts, in other instances lack of Ca may also produce contractions (255) but it seems best to consider calcium in its relation to potassium, since there appears to be an ionic balance between the two, additions of one ion having generally the same effect as withdrawal of the other (140), though the effects are very complex. It seems probable that the effects of citrate (234) may be due to removal of Ca ions.

The influence of the hydrogen ion has been investigated by Evans and Underhill (90), who were able to confirm the general statement of earlier workers that acids relaxed, and alkalies caused contraction, of the tissue. In these experiments the plain muscle was suspended in a saline solution in which the bicarbonate was replaced by a mixture of phosphates, so that the reaction could be altered, and would remain fairly stable while kept aerated by a stream of oxygen bubbles. Within the limits of hydrogen ion concentration which are generally considered as compatible with life, it was found that increase of H-ion concentration caused a relaxation of the tonus of the muscle. Rhythmic contractions were slowed and sometimes depressed. Excessive acidification led to death of the muscle in the *relaxed* state. Reduction of H-ion concen-

tration had the opposite effect of augmenting the tonus, and also quickened but depressed the rhythmic movements. Essentially similar effects have been reported by other workers (11, 97, 100, 125, 162, 205, 279).

When the muscle preparation had no appreciable tonus to begin with, addition of small amounts of acid had no effect, while alkalies had their usual effect of raising the tonus.

It seems that these effects are due to a direct action on the muscle tissue itself, and not in the first instance on the local nervous structures; at all events the same effects were seen in presence of 0.1 per cent of nicotine or 0.01 per cent of atropin, and also persisted from day to day if the tissue was kept on ice, for a period sufficient to ensure death of any ordinary nervous tissues. It was not possible to prepare any specimen of plain muscle which contained no nervous elements and at the same time possesses natural tonus in neutral Ringer's solution, so the question could not be directly answered.

An interesting phenomenon was seen when acid was added to a piece of plain muscle kept in a strongly alkaline phosphate Ringer solution; in this case there was not relaxation, but a transient contraction, or an increase of tonus. The explanation is that at very alkaline reactions (over pH 8) there is a reduction of calcium ion in the solution, and eventually deposition of calcium salts and no doubt a profound alteration in the surface properties of the cells in consequence of this; the tissue passes into a relaxed state although the reaction is alkaline, and when acid is added the calcium is suddenly liberated in the ionic state, and the contraction which follows is probably connected with some effect of this on the surface of the cells. In this connection it should be noted that Rona and Takahashi (226) have shown that the concentration of Ca ions depends on the hydrogen ion concentration according to the expression: $\frac{[Ca^{\cdot\cdot}]\,[HCO_3']}{[H^{\cdot}]} = k$. This phenomenon partly explains the very divergent results which have previously been obtained for the addition of acids and alkalies to plain muscle.

It is doubtful whether in any ordinary case the acid or alkali added to the surroundings actually penetrates into or produces alteration in the reaction of the cell cytoplasm; there is good reason to believe that such penetration if it occurs at all is very slow, with the possible exception of carbon dioxide, which in some respects behaves differently from other acids (90, 99, 143).

The effect of alteration of the hydrogen ion on the plain muscle of

invertebrates has been studied by Hogben (140) who finds that the crop of Aplysia and of Helix is much less affected by changes in reaction than is mammalian plain muscle. The crop of Aplysia shows an increase of tone when the acidity of the medium is gradually raised to about pH 5.0. When death occurs, however, it happens with the muscle in a fully relaxed state, as with mammalian tissue. With the heart of Helix, the effect of hydrogen ion concentration is bound up with the K/Ca ratio; when the K/Ca ratio is low, acidification leads to increase of tone and systolic standstill; when it is high, the reverse effect is obtained.

As regards the effect of other ions, there is little to be said. Of the negative ions, fluorides (218), iodides (218), and bromides (159, 218) are all said to have a stimulating action on plain muscle.

An able discussion of the effects of ions has been given by Hogben, who has pointed out that we are not yet in possession of suffcient data to enable us to form any definite generalisation on the subject. He draws attention to an important contribution of Rona and Petow (225) which may explain the peculiar action of potassium as compared with sodium.

They have put forward the suggestion that potassium forms complex ions with certain proteins, and Hogben suggests that the opposing action of the calcium and potassium ions may have some relation to this, and to the "externalisation of the lipoid phase" as suggested by Clowes.

Some observations of Andrus (8), and of Drury and Andrus (77) are of interest in connection with the effect of hydrogen ion on plain muscle. Andrus finds that the rhythm of the rabbit's auricle is reduced by increasing the hydrogen ion concentration of the perfusion fluid, the effect being more rapid when produced by phosphoric acid than by carbon dioxide. He assumes that the rate of development of the excitatory process depends on the difference in cH between the interior of the cell and the surrounding fluid, and this difference is less when carbon dioxide is used because it penetrates more readily. Evans and Underhill similarly found that the intestinal rhythm was slowed when the bath was made more acid. Andrus further found that in the auricle, the action of adrenaline or tyramine was reduced by rise of cH, and increased by fall of cH, whereas the (inhibitor) action of acetyl choline, or of vagus stimulation was increased by rise and reduced by fall of cH. On the rabbit's ileum, the action of acetyl choline was increased by *fall* of cH, and he infers that in each case the change in H-ion concentration primarily affects the susceptibility of the tissue to excitation or inhibition. In solutions of lower cH, the rate of development of spontaneous excitation is increased, and the tissue is more susceptible to artificial

stimuli, and less susceptible to inhibition. Evans and Underhill also found that the effect of pilocarpine on the small intestine was reduced when the cH was raised, which would be in agreement with this conception.

The rhythmic contractions of plain muscle. It is not known what is the ultimate cause of rhythmicity in any rhythmically functioning tissue, such as plain muscle, the heart, or the cells of the respiratory centre, but it appears fairly certain that any tissue which possesses excitability and a refractory period may, by at one time responding and shortly afterwards refusing to respond to the same stimulus, display rhythmicity (261).

A factor which might be responsible for the rhythmic contractions shown by plain muscle is the receipt by its cells of rhythmic stimuli from some local nervous mechanism, either ganglion cells or a nerve net. The view that the spontaneous contractions of the frog's stomach were neurogenic was held by Schultz, who believed that the nervous mechanism could be put out of action by application of 1 per cent atropin. Dixon, however, found that the contractions were not abolished by cocain up to 0.1 per cent on the outside or 0.5 per cent on the inside of the stomach, or by 0.3 per cent nicotine applied to the outer surface, although tissues poisoned in this way were unaffected by pilocarpine. That the muscle fibres themselves were not greatly injured by this treatment was shown by the fact that they contracted in response to barium chloride. It seems certain that the high concentrations of atropin used by Schultz were toxic to the muscle (285), while weaker solutions produced little effect. That atropin is unsuitable for the purpose is shown by the well-known pharmacological fact that after administration of atropin the effects of vagus or sympathetic stimulation on the movements of the stomach and intestine are unaltered. De Zilwa (281) found the same to hold for the retractor penis of the dog, and considers that Schultz's results are due to an effect of atropin on the muscle. Edmunds (80) shares this opinion as regards the retractor penis.

The small intestine has been much used in investigations on this question. Bayliss and Starling (15) believed that the peristaltic movements were neurogenic, but that the segmenting ones were myogenic, since they continued after the application of nicotine or cocaine. Hammett (125) also found rhythmic contractions to be unaffected by cocaine. Magnus (176) found that when the longitudinal coat, plus the Auerbach's plexus, was stripped off, it continued to show the segmenting movements, while his earlier work indicated that the circular coat,

devoid of nervous structures did not. This fundamental point was reinvestigated by Gunn and Underhill, who showed that it was possible, by avoiding damage to the muscle, to prepare strips of the inner layers of the circular coat which showed good rhythmic contractions. Such strips have been examined histologically by Underhill, who failed to find definite evidence of the presence of nerve cells.[5] Alvarez and Mahoney (6) have fully confirmed Gunn and Underhill's observations. It would thus seem that so far as the intestine is concerned, there is good evidence against the participation of the ganglion cells of Auerbach's plexus in the production of the rhythm.

Gross and Clark (116) have brought forward evidence from experiments on the effect of oxygen lack, which indicate the myogenic nature of the pendular contractions. According to Eiger (83), Pugliese (219), and many others, the spontaneous contractions in plain muscle are of neurogenic origin, and Tiegs (257) has recently expressed the opinion that even after a tissue has been deganglionated, the rhythm is dependent on the presence of the fine nerve net. Hryntschak (147) and Siccardi and Loredan (244) believe the movements to be myogenic.

In many invertebrates, it must be admitted (261) that the presence of nerve *cells* does not appear to be essential to the generation of spontaneous rhythmic contractions.

The most satisfactory evidence in favour of the myogenic nature of the spontaneous contractions is that afforded by the observations of Verzár (268); he found that the muscle cells of the chick amnion, which consist of a single layer of plain muscle cells devoid of nerve fibres, exhibit rhythmic contractions at the rate of about 16 per minute. An exactly similar observation has been made by Lewis and Lewis (167).

It must be admitted that in many varieties of plain muscle, the nervous structures take a form very similar to that of the primitive nerve net of invertebrates. In such creatures as medusa, sea-stars, etc., the rhythmic contractions of the muscle are believed to be in part a consequence of extension, which leads to an increase of tonus, and vice versa; this is viewed as the result of a fundamental property of the nerve net (261), and may be regarded as a simple type of reciprocal reflex similar, e.g., to that seen in the cat's tail, which responds to a stimulus by moving in such a way that the extended muscles contract.

[5] I understand from personal communications that Prof. H. S. Gasser has also examined such strips and found them free from nerve cells; his strips I believe showed strong contraction when muscarine or pilocarpine was added, but did not react to nicotine.

It would not be unreasonable, however, to suppose that the muscle itself shows a variation of excitability according to its length; if the longer muscle is the more excitable, then we should have a satisfactory explanation of the rhythmic contractions, in response to some constant stimulating condition.

The question arises whether such constant stimulus may arise from the presence, in the tissue itself, of exciting substances. Mansfeld (179) thinks that the excitant may be carbon dioxide. Or again, conjecturally, it may be lactic acid. Extracts of plain muscle and also of other tissues, have been shown by many investigators (e.g., 12, 78, 92, 133, 244, 250) to contain substances which excite contraction in plain muscle. Le Heux (134, 135, 136) has extracted choline from saline solutions which have been left for some time in contact with the serous coat of the intestine, and he and Magnus (175), Arai (9), Kühlewein (160) and Abderhalden and Paffrath (1) are of the opinion that choline acts as a "hormone" for the motor function of the alimentary canal. Le Heux is of the opinion that the choline acts on the Auerbach's plexus rather than on the muscle direct. Tezner and Turolt believe (255) that potassium is essential for the action of choline. Backmann (12) considers that the exciting substance in the extracts is not choline.

Zunz and Tysebaert (282) find that the exciting action on the intestine, of extracts of stomach and intestine, is not affected by atropine.

A similar substance is present in blood plasma and especially in serum, and is present in their alcohol extracts. Storm van Leeuwen and Zeydner (165) believe that the amount of the excitant is increased in the blood of asthma patients, but this is denied by Brabant. As regards the nature of the substance, Ponder (217) finds that its solubilities and properties do not agree with those of pituitrin, histamine or choline; Chistoni (56) admits the presence of choline, but inclines to the view that neurine is also present. The plain muscle stimulants in various body fluids have been studied by de Boer, Dreyer and Clark (21).

THE CONTRACTION CURVE OF PLAIN MUSCLE. The curve of contraction in plain muscle in response to a single stimulus resembles, at first sight, the one obtained from striated muscle; it differs, however, in being altogether much slower. The latent period, period of contraction and period of relaxation are all much longer, and are less constant. There are also differences in the properties of the muscle from different situations (5, 110). As the strength of stimulus is increased it is seen that the latent period becomes shorter, the height of contraction greater, and there is an increasing tendency to the establishment of tonus. The

variation in the length of latent period and height of contraction are seen very well with chemical as well as with electrical stimuli. The relation between concentration of drugs and their actions has been investigated by Storm van Leeuwen and Le Heux (166): they find that the effect of certain narcotics is in linear relation to the concentration at which they are presented, whereas the action of various exciting substances, mainly basic, varies asymptotically with the concentration of the drug in the medium, thus suggesting an adsorption phenomenon. I have also carried out some experiments with similar results.

Isometric contractions (33) can be obtained from plain, as from striated muscle; there seems to be little essential difference between

TABLE 5

Cat's ileum: stimulation by histamine 1 in 1 million

LENGTH	INITIAL TENSION	TENSION ON ISOMETRIC CONTRACTION	TENSION INCREMENT ON ISOMETRIC CONTRACTION
cm.	*grams*	*grams*	*grams*
3.2	0	50	50
3.7	2	68	66
4.2	30	90	60
4.45	50	105	55
4.7	85	131	46
4.95	137	168	31
5.2	186	209	23
5.2	162	193	31
4.95	50	112	62
4.7	20	75	55
4.45	18	65	47
4.2	15	48	33
3.7	0	30	30
3.2	0	3	3

them in this respect, except that one does not see the same development of great tensions in plain muscle, as in striated muscle. Brocklehurst finds (35) that at slightly greater initial lengths there is on contraction, a development of a greater tension, just as is the case with skeletal muscle. With further increase in initial length, the tension increment on contraction becomes less, the extension at which the optimal tension increment occurs being variable, and depending to some extent on the previous history of the muscle.

I am indebted to Doctor Brocklehurst for the following typical experimental results.

The differences in the figures for tensions at ascending and descending

initial lengths is due mainly to the after-extension and after-retraction of the tissue.

The onset of fatigue on repeated excitation is shown by progressive diminution in the height of the contractions. With the onset of fatigue, not only are the contractions smaller, but the propagation of a contraction wave in such structures as the ureter is less good, and the wave soon dies out. A fatigue curve for plain muscle sometimes shows a very striking contrast to one for voluntary muscle (118, 239); the latent period is greatly prolonged, and whole contraction and relaxation is *quickened*, and as fatigue progresses, the muscle passes into a state of relaxation, and not into a state of contracture. In striated muscle fatigue retards relaxation, in plain muscle it often hastens it. I have not always found these statements to hold good however.

Electrical changes. Very complicated records of the electrical changes in plain muscle have been published by several experimenters. In most cases a rhythmically contracting organ has been used and the changes associated with the rhythmic contractions recorded, e.g., ureter (Orbeli and Brücke (212)), intestine (Alvarez (4)), stomach (Richter (223)) etc. As an instance of these we may take the work of Orbeli and Brücke. By leading off from two points on the surface of the ureter *in situ*, they obtained curves which showed from 1 to 4 waves. Those which contained 4 waves, they explained as resulting from the superposition of inhibitory and excitatory waves, passing the leading-off points in the order inhibition-excitation (diphasic)—inhibition: this gives waves in order $+\ (-\ +)-$, the part in parentheses being the diphasic excitation wave, while the diphasic inhibition wave is split into two parts, one before and one after the excitation wave. It is difficult to follow how such a sequence could possibly occur and give this result. My own experiments on the heart of *Helix pomatia* (85) show that the electrical change is of the same nature as that seen in the propagation of other excitatory disturbances in other tissues, namely diphasic. The complex ureter tracings and electrogastrograms which have been obtained are, I think, due partly to the complex direction of the muscular coats, and to other extraneous causes, such as displacement of electrodes, or of fluid beneath them. In the more simply arranged muscle of the frog stomach, H. H. Funke (106) has recently found that the electrical changes are also of diphasic type as a rule; they are slow and prolonged, have a gross latent period of 3 to 5 seconds and last for a shorter time than the mechanical changes associated with them.

Heat production. We have next to no information concerning the

heat production during contraction in plain muscle. Some experiments by Snyder (247, 248) and by Bernstein (17) indicate that the heat production is small and that the heat is slowly developed during the contraction. We are, however, in almost complete ignorance of the thermal relations of plain, as contrasted with striated, muscle.

OSMOTIC RELATIONS. It is generally known that striated muscle fibres swell, shorten and pass into rigor when placed in distilled water, and shrink when placed in hypertonic solutions (107). It is believed that the fibres of striated muscle are surrounded by a semipermeable membrane, permeable to water, impermeable to most salts, and that thus the salt content of the fibres is kept different from that of the surrounding fluids. Theories of muscular contraction based on changes of osmotic pressure in the fibrils or in parts of them have often been put forward.

Meigs and Ryan (189), from a study of the salts of plain muscle, and of the behavior of the tissue when placed in hypotonic saline solutions, consider that there is no semipermeable membrane around the cell because the gain in weight of the tissue in hypotonic saline solutions is so much slower than with skeletal muscle. Yet the salts seem to be incapable of diffusing out of the tissue, and it is therefore assumed that they are present in some non-diffusible form. If we assume there is to be no semi-permeable membrane, the change in volume of the cells in hypotonic solutions is attributable to imbibition of water by the colloidal gels of the tissue, and that the salts are non-diffusible because they are held on to the proteins by adsorption.

The osmotic relationships of smooth muscle have been studied by Meigs (188, 192, 197), who has compared them with those of striated muscle of the same animal (frog); these investigations have been repeated and extended in my laboratory by Dr. S. W. F. Underhill. The following table shows in summary, a comparison between their results.

The results show the rough average changes in weight when the tissue has been steeped in the solutions named until the weight has become approximately constant, say after 3 or 4 hours.

It appears from these results that though striated muscle swells more readily at first in hypotonic solutions than the plain muscle, ultimately, with more dilute solutions, or with water, the swelling of the plain muscle is much the greater. Further, the swelling in very dilute acids or alkalies is not clearly seen in smooth muscle, but when the pH of the solution falls below 3.5 or rises above 10, then rapid and extensive swelling occurs. It seems quite certain that these phenomena could be

satisfactorily explained as due to imbibition of water. Underhill has also examined the changes in length of the tissues steeped in these solutions, and found, as Meigs did, that very often swelling is accompanied by lengthening, and *vice versa;* but his conclusion is quite opposed to that of Meigs in one important respect, viz., that there is no necessary relation between length and extent of swelling. Thus acids and alkalies between pH 4 and pH 10 cause maximal alterations of length, but quite small changes in the weight of the muscle, while water, which causes such large increase of weight has a small or variable effect on the length.

TABLE 6

Changes in weight and length of smooth and striated muscle under different conditions

TREATMENT	MEIGS		UNDERHILL	
	Striated	Plain	Plain (Wt.)	Plain (Length)
	per cent	*per cent*	*per cent*	
Placed in Ringer.....	±5	+30	±10	+ 50 per cent at once (tonus)
Ringer, pH 4 to 10.....			±10	+ 50 per cent at once (tonus)
Ringer pH below 3.5 or above 10.5.....			+ 6 to 100	in acid + 30 per cent in alkali −30 per cent
Hypotonic Ringer (1:1)	+15	Very little		Usually lengthens
Ringer (1:5).....			+25	
Hypertonic R.....	−20	−20	−25	Shortens
Water.....	+60	+140	+120	Shortens 10 per cent or lengthens up to 30 per cent according to initial tonus
Acidified Ringer (lactic acid).....		Very little		

It seems wrong, therefore, to conclude, as Meigs did, that increase in the size of the muscle cells is a fundamental change which always accompanies, and possibly causes an increase in length. We must conclude that the fundamental alteration in the cell which leads to shortening is, as with striated muscle, a change in the elastic properties of the contents of the cell, and not mere inflation or shrinkage. A cell may shorten or lengthen without change of volume, or change its volume without altering its length appreciably. Length of a plain muscle cell, as has been said before, is the resultant of two things, an extending force and

the "tonus" or resistance to deformation of the cell; these are balanced, and change in length can only result from a temporary disturbance of this state of balance.

THE METABOLISM OF PLAIN MUSCLE, AND THE CAUSE OF CONTRACTION. *Relation to oxygen usage.* The rate at which oxygen is used by plain muscle, is (59, 60, 88), as already stated, rather slow, but shows no increase during the maintenance of tonus. Certain facts make it clear, however, that oxygen plays an important part in the contractile (or recovery) process. A constant supply of oxygen is necessary for the maintenance of the tonic state, and if plain muscle is deprived of oxygen (116, 145, 200), though it still remains capable of contraction, tonus cannot be sustained. This is seen for instance when cyanides (86, 128, 204), are added to the bath in which a preparation is suspended, for the addition of cyanides lowers the oxygen consumption very considerably (88). The same kind of result is obtained when the oxygen bubbling through the saline solution in the bath is replaced by pure nitrogen. In both cases tonus, whether natural, as in the cat's uterus, or induced by a drug, as in the guinea pig uterus or intestine, is lost when the oxidative changes are stopped, but returns at once when oxygen is readmitted. The natural inference, from what we know of the metabolism of striated muscle, would be that there has been in absence of oxygen, an accumulation of end-products, which has caused the relaxation. In support of this we have the following facts: 1, During the onset of fatigue, the muscle often passes into an increasingly relaxed state. 2, In heat paralysis in spite of the fact that oxygen utilisation is at a maximum, full relaxation with loss of contractility occurs (Evans, 87). 3, After a period of oxygen lack, there is, on re-admission of oxygen, an accelerated oxygen usage (88). Thus a guinea pig uterus which used 0.11 cc. oxygen per gram hour under conditions of normal oxygen supply, used 0.21 cc. per gram hour in the early recovery from a 2-hour period of anoxybiosis.

It is difficult to understand the prompt recovery of tonus in a muscle which has lost its tone by deprival of oxygen. It appears to be much too sudden to be explicable as due to the operation of restitutive oxidative recovery processes. I am inclined to regard it as due to some surface effect, such as an alteration of permeability.

Reducing power. Tsubura (259) has investigated the reduction of methylene blue by plain muscle under anaerobic conditions, and found that in most respects it conforms qualitatively with skeletal muscle, though the reduction is much slower. When well washed with water,

the reducing power is lost, but is partially restored by addition of succinates, or by boiled extract of plain muscle. The extract of plain muscle, even after boiling, shows considerable power of reducing methylene blue in vacuo, which is explained by the further fact that the extract gives the nitroprusside reaction for glutathione very strongly, more strongly in fact than a similar extract of striated muscle. For the same reason the extract of plain muscle can restore the reducing power to washed striated muscle better even than the striated muscle extract does. The reduction is not inhibited, but is in fact slightly accelerated by presence of cyanides. The reducing power of washed plain muscle is slightly restored by lactates, but not by acetates or formates. m-dinitrobenzene is reduced to the yellow nitraniline more readily by plain than by striated muscle.

The formation of lactic acid. I have recently shown (89) that lactic acid is formed in plain muscle under conditions similar to those which lead to its formation in skeletal muscle. Thus for example, it is formed when the muscle is subjected to repeated stimuli, or when it is minced in an alkaline solution, as the following examples illustrate.

Per cent lactic acid: stomach of tortoise

TREATMENT	PER CENT LACTIC ACID	
	(1)	(2)
Control	0.029	0.013
Stimulated for 1 hour	0.085	
Stimulated 3½ hours		0.086
Control	0.025	
Minced in 0.8 per cent $NaHCO_3$ and left 2½ hours	0.087	
Minced in m/15 Na_2HPO_4—2.5 hours	0.078	
Minced in m/15 NaH_2PO_4—5¾ hours	0.058	
Minced m/15 Na_2HPO_4—19.5 hours	0.165	
Minced in Na_2HPO_4 plus arsenate 24 hours	0.13	

The experiments show that there is undoubted formation of lactic acid in contraction, and also that the survival production of lactic acid is accelerated by immersion in a solution of alkaline reaction. The maximal lactic acid production which could be obtained, even in the presence of arsenate and phosphate, was only of the order of 0.13 to 0.16 per cent, and we must conclude that this is due to the fact that the amount of lactic acid precursor is much smaller than in striated muscle.

Lactic acid is also formed at an accelerated rate when the tissue is deprived of oxygen, or immersed in a cyanide solution, and I have also been able to show that if the asphyxiated tissue is returned to oxygen, there is a removal of the lactic acid. The following table illustrates this.

It is evident that there must be a continuous removal of lactic acid from the tissue when it is kept in oxygen, and I have been able to show that the recovery process by which this is effected is in all probability similar to that which takes place in skeletal muscle. In one experiment, for instance, there was in nitrogen an increase of 0.67 mgm. of lactic acid per gram of tissue in 6 hours, while when in oxygen there was only an increase of 0.12 mgm. per gram; the presence of oxygen had thus in 6 hours prevented the formation of 0.55 mgm. of lactic acid per gram of tissue.

TABLE 7

Effect of oxygen lack on lactic acid

Tissue fresh (control)	= 0.049 per cent lactic acid
Tissue 20.5 hours in O_2	= 0.061 per cent lactic acid
Tissue in nitrogen for 80 minutes	= 0.078 per cent
Tissue in nitrogen for 6½ hours	= 0.118 per cent
Tissue in nitrogen for 20 hours	= 0.124 per cent
Tissue in nitrogen 6½ hours, then in O_2 13.5 hours	= 0.096 per cent lactic acid
Tissue in nitrogen 20 hours, then in O_2 7 hours	= 0.102 per cent lactic acid
Tissue in oxygenated Ringer 22½ hours	= 0.038 per cent lactic acid
Tissue in Ringer plus n/1000 NaCN for 22½ hours	= 0.14 per cent lactic acid

This amount of lactic acid would require 410 mm.^{3} of oxygen to oxidise it, and if under aerobic conditions the lactic acid were removed entirely by oxidation, the resting tissue should use 410 mm.^{3} oxygen in 6 hours; but direct experiment showed that actually only 123 mm.^{3} of oxygen were used in that time. That is to say that only about one-third of the lactic acid is oxidised (actually 1/3.35), the remainder being removed in some other form. This so closely resembles what happens in skeletal muscle, that, is seems almost certain that what has happened to the remaining two-thirds of the lactic acid is a restoration to the lactic acid precursor, presumably glycogen. Destruction of added glycogen by plain muscle was demonstrated by Saiki (232).

Experiment showed that the lactic acid arises in plain muscle from carbohydrate, and chiefly from glycogen. The following experiments illustrate this:

Lactic acid, glycogen and soluble carbohydrate in plain muscle

	RESTING CONTROL	TISSUE IN n/1000 CYANIDE 19 HOURS	DIFFERENCE
	per cent	*per cent*	*per cent*
Lactic acid	0.015	0.116	+0.101
Glycogen	0.139	0.038	−0.101
Soluble carbohydrate	0.064	0.050	−0.014
Total carbohydrate (as glucose)	0.214	0.091	−0.123

Effect of asphyxia and recovery

	(1)	(2)	(3)	(4)	(5)
	REST CONTROL	TISSUE 4½ HOURS IN NITROGEN	DIFFERENCE (1) AND (2)	TISSUE 4½ HOURS IN NITROGEN AND 19 IN OXYGEN	DIFFERENCE (2) AND (4)
Lactic acid	0.016	0.092	+0.076	0.079	−0.013
Glycogen	0.151	0.100	−0.051	0.122	+0.012
Soluble carbohydrate	0.172	0.141	−0.031	0.132	−0.009
Total carbohydrate as glucose	0.334	0.249	−0.085	0.253	+0.004

The experiments show that as the lactic acid increases the glycogen diminishes: during recovery, the reverse happens. The position of the soluble "carbohydrate" is less clear.

Rigor. Many investigators (100, 129, 138, 177, 178, 203, 211, 233, 236, 281, 286) studied the effect on plain muscle of conditions which in striated muscle would lead to rigor mortis. As a result of their studies, and from my own experience, I incline to agree with Saxl (236), Morita (203), de Zilwa (281) and several others, who believe that rigor does not definitely occur in plain muscle when it is allowed to die, or is killed by heat, chloroform, or injury. At all events, definite shortening is not regularly seen under these conditions, but more frequently lengthening.

Nevertheless, when subjected to such treatment as would produce rigor in skeletal muscle, there is in plain muscle an accelerated production of lactic acid, whether there be shortening of the tissue or not (89).[6] Formation of acid has also been shown by Hirsch (138), though Winter and Smith (278) deny any increase in phosphates on death.

Theories of contraction. The immediate cause of the shortening of

[6] An exception to this is the action of caffeine, which *retards* lactic acid production in plain muscle.

plain muscle is not known. According to Meigs (192) it is due to a loss of water from the cells; and although the experiments of Hopkins (144) support this idea, much recent evidence does not. Hogben (140) discusses various possibilities, but is of the opinion, with which I wholly agree, that at present it is impossible to form any decision. Evans and Underhill (90) thought that the effect of acids indicated a different mechanism in plain muscle from that in striated, but Hogben suggests some fundamental objections to this conclusion. Nevetheless, I think that the effect of acids on plain muscle is of interest when viewed from the standpoint of Andrus (8) according to which the difference between the H-ion concentration of the inside and outside of the cell is a determining factor. Viewed from this standpoint, an important factor would be the physico-chemical state of the cell boundaries.

On the other hand, there is evidence that fundamental changes occur in the cytoplasm itself during contraction: the decrease in transparency, the changes in refraction, and the movements of the cytoplasm suggest the possibility that there is an alteration in the fluidity of the cytoplasm, such as would be compatible with the change of its colloids from the sol to the gel condition. The facts of anaphylaxis in plain muscle which have been studied by Dale (71, 73) lend support to this idea that there is some change in physical state which is closely connected with, if not primarily responsible for, the act of contraction.

In searching for the ultimate cause of this change, whatever it may be, investigators have almost invariably turned for guidance to the phenomena of striated muscle, and have supposed that there must be a production of lactic acid which is responsible for the contraction changes. Although I have been able to show that lactic acid is indeed produced by plain muscle under conditions similar to those which lead to its formation in striated muscle, I cannot feel that this demonstration has brought us much nearer to an understanding of the fundamental cause of contraction. For instance, cooling the tissue often causes contraction, whereas paralysis is seen on warming, which accelerates lactic acid production. It could of course be assumed that the lactic acid was generated at a certain place in the cell, produced its effects, and then was either neutralised (Meigs, 192) or removed by diffusion or oxidation. Tiegs (257) has assumed the production of lactic acid and of an unknown basic substance at the centre of each cell; in short contractions both are liberated simultaneously, and diffuse to the contractile material, the acid reaching it first, followed by the base; in tonus, only the acid is liberated. There is however, no experimental support for such an hypothesis.

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